*Kidney International, Vol. 54 (1998), pp. 2064–2080*

# A simultaneous study of the metabolism of apolipoprotein B and albumin in nephrotic patients[1]

**THOMAS DEMANT, CHRISTOPH MATHES, KATJA GÜTLICH, ANDREA BEDYNEK, HJALMAR B. STEINHAUER, THOMAS BOSCH, CHRISTOPHER J. PACKARD, and GRAHAM L. WARWICK**

*Institut für Klinische Chemie und Medizinische Klinik I, Klinikum Grosshadern, Ludwig-Maximilians-Universität, München, and Carl-Thiem-Klinikum, II. Medizinische Klinik, Cottbus, Germany; Leicester General Hospital, Leicester, England, and Institute of Biochemistry, Glasgow Royal Infirmary NHS Trust, Glasgow, Scotland, United Kingdom*

**A simultaneous study of the metabolism of apolipoprotein B and albumin in nephrotic patients.**

*Background.* The nephrotic syndrome is characterized by proteinuria, hypoalbuminemia and hyperlipidemia. Despite intensive research it is not clear at present what the causal links are between these pathological findings.

*Methods*. Stable isotope labeled amino acid tracer kinetic analysis was used to simultaneously investigate the metabolism of four apolipoprotein B-containing lipoproteins ($VLDL_1$, $VLDL_2$, IDL and LDL) and albumin in seven patients with nephrotic syndrome and marked hypercholesterolemia, in two additional nephrotic patients with concomitant renal failure and mixed hyperlipidemia, and in a matched group of normolipidemic controls.

*Results*. Increased concentrations of $VLDL_2$, IDL and LDL were due to (*a*) impaired $VLDL_2$ and IDL delipidation, (*b*) reduced LDL catabolism, and (*c*) a trend towards an increased rate of total apolipoprotein B production. The rate of fractional albumin elimination was three times higher in patients than in controls and the rate of albumin synthesis was increased by 45%. No correlations were detectable between rates of apolipoprotein B production and the rate of albumin synthesis.

*Conclusions*. The results of this study suggest that hyperlipidemia in nephrotic syndrome is predominantly the result of delayed lipoprotein delipidation and catabolism. There is no evidence that it is driven by a general increase of the rate of hepatic protein synthesis.

Hyperlipidemia with increased cholesterol and triglyceride levels in plasma is a typical feature of the nephrotic syndrome [1]. Usually, low density lipoprotein (LDL) concentrations are increased well above the normal range, but very low density (VLDL) may also be increased, particularly in cases of marked hypoalbuminemia or in the presence of concomitant renal failure [2]. In contrast, high density lipoproteins (HDL) have been reported to be either normal or decreased [3]. In general, VLDL is secreted by hepatocytes as a lipoprotein consisting mainly of triglycerides and the apolipoprotein B-100. By stepwise delipidation and lipid exchange VLDL is transformed in the plasma compartment into cholesteryl ester rich intermediate density lipoproteins (IDL) and eventually into LDL. While the lipid moiety of these lipoproteins is extensively modified and remodeled in this process, the apo B-100 molecule is preserved and stays with a lipoprotein particle from its nascent state until it is finally eliminated from the circulation either by binding to the LDL receptor or through receptor-independent mechanisms. For the general population it is now well established that increased LDL concentrations are correlated with an increased risk for coronary heart disease. The increased levels of apo B containing lipoproteins, particularly in the density range of IDL and LDL, are a major reason for the accelerated atherosclerosis observed in patients with nephrotic syndrome [4, 5].

The disturbance of apolipoprotein B metabolism in the nephrotic syndrome is thought to be caused by increased secretion of apo B containing lipoproteins either alone [6, 7] or in combination with impaired lipoprotein clearance from the plasma [8–11]. Studies in humans [6–12], experimental animals [13–17], and in *in vitro* systems [18–20] have produced evidence for both pathogenic principles. However, it is still a matter of controversy whether increased synthesis or reduced catabolism are the leading cause of hyperlipidemia in nephrotic patients. One common hypothesis suggests that hypoalbuminemia due to urinary protein losses results in increased hepatic albumin synthesis, which in turn leads to a generalized increase in hepatic protein synthesis including apolipoprotein B production [14, 21]. Alternatively, it is assumed that apo B

[1] Dedicated to Professor Dr. Dietrich Seidel, Head of the Institut für Klinische Chemie, Klinikum Grosshadern, LMU München, on the occasion of his 60th birthday.

**Key words:** albumin, stable isotopes, kinetic analysis, nephrotic syndrome, hyperlipidemia, apolipoprotein B.

Received for publication August 20, 1997
and in revised form June 29, 1998
Accepted for publication July 1, 1998

**Table 1.** Nephrological diagnosis and non-lipid parameters of patients with nephrotic syndrome

| Name | Creatinine *mg/dl* | Urinary protein *g/24 h* | Urinary albumin *g/24 h* | Nephrological diagnosis *based on histology* | Duration of disease *years* |
|---|---|---|---|---|---|
| WM | 1.2 | 16.3 | 14.0 | membranous glomerulonephritis | 4 |
| KF | 1.2 | 16.0 | 14.0 | membranous glomerulonephritis | 2 |
| JO | 0.9 | 11.4 | 10.1 | membranous glomerulonephritis | 2 |
| HA | 1.1 | 9.6 | 9.6 | membranous glomerulonephritis | 1 |
| MC | 0.8 | 12.0 | 9.3 | membranous glomerulonephritis | 5 |
| EA | 0.4 | —[a] | —[a] | focal segmental glomerulosclerosis | 6 |
| ED | 0.7 | 7.3 | 4.6 | focal segmental glomerulosclerosis | 8 |
| Mean ± SD | 0.9 ± 0.3 | 12.1 ± 3.5 | 9.5 ± 3.3 | | |
| HS | 2.7 | 5.4 | 5.4 | chronic glomerulonephritis (no histology) | 4 |
| KD | 2.4 | 4.1 | 3.9 | focal segmental glomerulosclerosis | 1 |

[a] Not available from the time of turnover study

**Table 2.** Lipoproteins and biometric data of patients with nephrotic syndrome

| Name | Sex | Age *years* | Body wt *kg* | Height *m* | Albumin *g/dl* | Total chol. *mg/dl* | Triglycerides *mg/dl* | VLDL-chol. *mg/dl* | LDL-chol.[a] *mg/dl* | HDL-chol. *mg/dl* |
|---|---|---|---|---|---|---|---|---|---|---|
| WM | m | 50 | 90 | 1.76 | 2.8 | 292 | 440 | 77 | 182 | 33 |
| KF | m | 56 | 102 | 1.78 | 2.6 | 282 | 202 | 38 | 182 | 62 |
| JO | m | 62 | 70 | 1.67 | 2.8 | 413 | 127 | 25 | 317 | 72 |
| HA | m | 52 | 73 | 1.59 | 2.9 | 500 | 182 | 36 | 373 | 90 |
| MC | m | 32 | 76 | 1.80 | 2.7 | 292 | 163 | 32 | 210 | 49 |
| EA | f | 21 | 65 | 1.66 | 1.7 | 365 | 105 | 21 | 302 | 42 |
| ED | f | 36 | 56 | 1.68 | 2.5 | 259 | 137 | 27 | 187 | 46 |
| Mean ± SD | | 44 ± 15 | 76 ± 15 | 1.71 ± 0.08 | 2.6 ± 0.4 | 343 ± 88 | 194 ± 114 | 37 ± 19 | 250 ± 79 | 56 ± 20 |
| Neph. vs. Cont. | | NS | NS | NS | $P < 0.001$ | $P < 0.005$ | $P < 0.020$ | $P < 0.020$ | $P < 0.007$ | NS |
| HC | m | 62 | 90 | 1.80 | 4.1 | 248 | 419 | 75 | 146 | 28 |
| KD | m | 45 | 93 | 1.81 | 4.7 | 263 | 384 | 81 | 149 | 32 |

[a] Abbreviations are: chol., cholesterol, Neph. vs. Cont., nephrotics versus controls; LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein. "LDL" is determined by standard laboratory procedures in line with the Lipid Clinics Research protocol (for details see [24]). It comprises the LDL- and IDL-fractions ($S_f$ 0-20) prepared by cumulative gradient ultracentrifugation.

production is enhanced by a reduction of plasma oncotic pressure due to low plasma albumin concentrations [18]. As a result, one would expect to find either rates of albumin synthesis or albumin plasma levels to be correlated with rates of apolipoprotein B production in hyperlipidemic nephrotic patients.

Until recently the metabolism of apo B-containing lipoproteins and of albumin could not be investigated at the same time in the same individual. Therefore, virtually all studies published thus far are confined to the kinetic analysis of either lipoprotein or albumin metabolism and sought to draw conclusions with regard to the other component by monitoring surrogate metabolic parameters such as plasma concentrations [8, 22, 23]. By the combined use of stable isotope labeled amino acid tracers, sensitive mass spectrometric analysis and multicompartmental kinetic modeling, it has become possible to study the metabolism of two or even more proteins simultaneously in one subject [24–26].

Here we report the results from a metabolic study in nine nephrotic patients where deuterated leucine was used as a tracer for endogenous labeling of newly synthesized proteins in order to investigate the putative links between albumin and apolipoprotein B metabolism.

## METHODS

### Subjects

Seven patients participating in this study were recruited from out-patient clinics of the nephrology units of three different hospitals. They suffered from nephrotic syndrome for one to eight years and fulfilled the following selection criteria: (*a*) proteinuria $> 5.0$ g/day, (*b*) serum creatinine $<$ 1.3 mg/dl (115 $\mu$mol/liter), and (*c*) plasma cholesterol $>$ 250 mg/dl (6.5 mmol/liter). Details of their histological diagnoses, serum creatinine concentrations and urinary protein losses are listed in Table 1. Biometrical data together with lipoprotein profiles are given in Table 2. Two other patients with nephrotic syndrome (HS and KD) who did not fulfill the above selection criteria due to moderate renal insufficiency were also included in the present study. However, results from their metabolic trials were recorded separately and were not pooled with those of the study group. Patients received diuretics (furosemide plus amiloride or hydrochlorothiazide, all except MC) and

**Table 3.** Lipoproteins and biometric data of normal controls

| Name | Sex | Age *years* | Body wt *kg* | Height *m* | Albumin *g/dl* | Total chol. | Triglycerides | VLDL-chol. | LDL-chol.[a] | HDL-chol. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *mg/dl* | | | | |
| BG | m | 30 | 83 | 1.80 | — | 175 | 89 | 19 | 104 | 52 |
| CE | m | 29 | 61 | 1.75 | 4.8 | 202 | 95 | 20 | 136 | 47 |
| MM | m | 30 | 81 | 1.78 | — | 215 | 80 | 27 | 118 | 70 |
| BJ | m | 33 | 60 | 1.70 | — | 232 | 96 | 15 | 161 | 42 |
| RB | m | 48 | 106 | 1.86 | 4.7 | 194 | 99 | 22 | 130 | 56 |
| CS | m | 29 | 75 | 1.81 | 5.2 | 155 | 122 | 23 | 78 | 54 |
| WS | m | 43 | 83 | 1.87 | 5.0 | 219 | 103 | 19 | 142 | 58 |
| JD | f | 46 | 60 | 1.72 | 4.2 | 175 | 60 | 12 | 115 | 48 |
| GH | f | 26 | 57 | 1.68 | 5.2 | 220 | 40 | 9 | 131 | 80 |
| Mean ± SD | | 35 ± 8 | 74 ± 16 | 1.78 ± 0.07 | 4.9 ± 0.4 | 199 ± 26 | 87 ± 24 | 18 ± 6 | 124 ± 24 | 56 ± 12 |

Abbreviations are given in Table 2.
[a] See footnote [a] of Table 2

angiotensin-converting-enzyme inhibitors (WM, KD, JO). None of them was on immunosuppressive therapy and lipid lowering drugs had been withdrawn for more than one month prior to metabolic studies. A control group of nine normolipidemic subjects was selected from laboratory staff and medical students. Lipoprotein profiles and biometrical data are presented in Table 3. Body mass indices ($kg/m^2$) in patients and in controls were not significantly different ($26.0 \pm 4.2$ and $23.3 \pm 3.6$ $kg/m^2$, respectively; $P = 0.183$). Throughout the study, patients and controls were asked to continue their normal diets. Three patients and five controls prepared seven-day weighed food intake protocols that were analyzed by a dedicated computer program (Prodi; Nutriscience, Karlsruhe, Germany).

All subjects participating in this study gave informed consent. The study met the requirements of the Ethics Committees of the Klinikum Grosshadern in Munich, the Carl-Thiem-Klinik in Cottbus, the Leicester General Hospital in Leicester and the Royal Infirmary in Glasgow.

### Turnover protocol

The protocol employed has been described in detail elsewhere [24]. Subjects were fasted for 12 hours overnight and at 8 a.m. the tracer amino acid, $d_3$-leucine (Cambridge Isotopes, Woburn, MA, USA), was given either as an intravenous bolus injection (6.0 mg/kg body wt; subjects: WM, JO, HS, KD, BG, BJ, WS, JD, GH) or as a primed constant infusion (that is, a bolus of 0.6 mg/kg followed by 0.6 mg/kg/hr for 10 hr; subjects: KF, HA, MC, EA, ED, CE, MM, RB, CS). Both methods of tracer administration have been tested previously and found to provide identical metabolic parameters [24, 25]. Plasma samples (10 ml blood collected in EDTA) were collected for apolipoprotein B, albumin and plasma free leucine analysis immediately before tracer administration, at multiple time points throughout the first 12 hours of the study, and thereafter, daily in the fasting state for 12 days. A light meal was offered 10 hours after tracer injection.

### Lipoprotein isolation and preparation of apo B

$VLDL_1$ ($S_f$ 60 to 400), $VLDL_2$ ($S_f$ 20 to 60), IDL ($S_f$ 12 to 20) and LDL ($S_f$ 0 to 12) were prepared from 2 ml plasma by cumulative gradient ultracentrifugation as previously described [24]. Briefly, 2 ml plasma were adjusted to a density of d = 1.118 g/ml by addition of solid NaCl and built into a discontinuous six-step salt gradient ranging from d = 1.0988 to 1.0588 g/ml. After the following centrifugational runs at 23°C in a Beckman SW40 rotor, lipoprotein preparations were collected from the surface of the gradient: $VLDL_1$ (1.0 ml, replaced by 1.0 ml of d = 1.0588 g/ml solution), 39,000 rpm, 1.63 hours; $VLDL_2$ (0.5 ml), 18.500 rpm, 15.68 hours; IDL (0.5 ml), 39,000 rpm, 2.58 hours; and LDL (1.0 ml), 30,000 rpm, 21.17 hours. Prior to delipidation LDL preparations were tested for contaminations by lipoprotein(a), which when detectable was removed quantitatively by affinity chromatography using immobilized wheat germ agglutinine [27]. Apo B was precipitated from lipoprotein fractions by the addition of an equal volume of isopropanol, the resulting pellet was delipidated with ethanol:ether (3:1), dried with ether and hydrolyzed at 110°C for 20 hours in the presence of 0.5 to 1.0 ml 6 N HCl, which was subsequently removed by evaporation in a vacuum concentrator centrifuge [24]. Lipids were determined by routine laboratory tests and protein was measured by the Lowry method as previously described. Apo B plasma pools were derived from the apo B content that was calculated as the difference between total and isopropanol-soluble protein, and an estimate of the plasma volume (4% of the body wt). The apo B masses of each of the lipoprotein fractions were corrected for experimental losses by comparing the total cholesterol recovered in the four fractions obtained by cumulative gradient ultracentrifugation, with the non-HDL cholesterol value determined in native plasma. The fractional leucine content of apo B was taken as 0.1212 g/g [24]. Lp(a) was

quantified as Lp(a) total mass by an ELISA technique (Immuno GmbH, Heidelberg, Germany).

### Albumin preparation

Albumin was prepared from plasma by affinity chromatography as described by Travis and Pannell [28]. EDTA-plasma was diluted 1:10 with 0.05 M Tris, 0.10 M KCl (pH 7.0) and 3 ml were applied on a 2 ml column containing Blue-Sepharose (Pharmacia, Upsala, Sweden). Non-binding plasma components were eluted with 3 × 2 ml 0.05 M Tris, 0.10 M KCl (pH 7.0) and subsequently albumin was desorbed with 0.05 M Tris, 1.50 M KCl (pH 7.0). It was transferred into 0.10 M ammonium acetate (pH 7.0) by passage over a NAP-5 column (Pharmacia, Upsala, Sweden), dried in a vacuum concentrator centrifuge and subsequently hydrolyzed as described above. Immunoelectropheresis of the desorbed protein fraction against anti-human-plasma antiserum (Behring, Marburg, Germany) showed a single band in the albumin position and no additional protein contamination (data not shown). From data in the literature a value of 0.1205 g/g for the leucine content of albumin was adopted [29]. Albumin in native plasma and in urine was determined by a nephelometric routine laboratory procedure (Behring, Marburg, Germany).

### Free amino acid preparation from plasma

Proteins were precipitated from 1 ml plasma by adding 1 ml trichloroacetic acid (10%) and amino acids prepared from the supernatant by cation exchange chromatography using 2 ml columns filled with Dowex AG-50W-X8 resin ($H^+$-form, 50 to 100 mesh; Biorad, Richmond, CA, USA). The amino acids that bound to the resin were desorbed by 4 M $NH_4OH$, which was subsequently removed by evaporation in a vacuum concentrator [30]. The samples were dissolved in a small volume of 1 M HCl, transferred into microvials and dried again ready for derivatization.

### Leucine tracer/tracee analysis by quadrupolar GC-MS

Amino acids derived from apo B, from albumin or from plasma were transformed into tert-butyl-dimethyl-silyl (TBDMS) derivatives by incubation with 50 $\mu$l of a freshly prepared 1:1 mixture of N-methyl-N-(tert-butyl-dimethyl-silyl)-trifluoro-acetamide (MTBSTFA; Fluka, Buchs, Switzerland) and acetonitrile in crimped microvials at 80°C for 20 minutes. Enrichments were determined immediately by gas chromatography mass spectrometry using a quadrupolar GC-MS instrument (Trio 1000; Fisons, Manchester, UK).

A detailed description of the method used for GC-MS analysis has been published elsewhere [24]. The gas chromatograph was equipped with a DB1701 capillary column (J&W, Folsom, CA, USA) operated at 110°C for one minute after sample injection, followed by an increase of temperature of 20°C/min up to 280°C. The mass spectrometer was used with electron impact ionization ($EI^+$). Leucine ion mass fragments were monitored and quantified in the selective ion recording (SIR) mode at mass-to-charge ratios (m/z) 277, m/z 276 and m/z 274. From these measurements the specific isotopic enrichment (E) and the leucine tracer/tracee ratio (Z) were calculated by the following formulas [31]:

$$E = (R - R_N)/[(1 + R) \cdot (1 + R_N)] \quad (1)$$

where R is the m/z 277 to m/z 274 ratio for the enriched sample and $R_N$ is the equivalent ratio for naturally occurring leucine ($R_N = 0.01697$; $N = 10$).

$$Z = E/(E_I - E) \quad (2)$$

where $E_I$ is the isotopic abundance of the infused tracer that was determined to be 0.998.

### Kinetic analysis and multicompartmental modeling

Typical examples for the time courses of leucine tracer/tracee ratios measured in the four apo B-containing lipoproteins and in albumin are shown in Figures 1 and 3. They were analyzed using a computer program for simulation, analysis and modeling (SAAM II, version 1.1.1; SAAM Institute, Seattle, WA, USA). Kinetic analysis performed on the basis of a metabolic model resulted in the determination of transfer rate coefficients ($k_{i,j}$) and masses ($Q_i$) for apolipoprotein B or albumin compartments. Values for $k_{i,j}$ describe the transfer of material from compartment j to i as fraction of pool j per unit of time. $Q_i$ quantifies the mass content of compartment i. Individual transfer rate coefficients ($k_{i,j}$) were summarized to provide rates of protein secretion, transfer (where applicable) and catabolism (Table 4). Kinetic parameters were usually determined with a fractional SD (FSD) of less than 0.10. In the few instances were FSD values were greater than 0.50 parameters were fixed at a calculated level. In all studies simulation calculations were performed until convergence between curves for observed and calculated tracer/tracee ratios was reached and further iterations were aborted by SAAM. Individual $k_{i,j}$ and $Q_i$ values and the appropriate FSD values are given in **Appendices I and II**.

The model used for the quantitative analysis of apo B metabolism as depicted in Figure 2A has been described in detail elsewhere [24]. Its basic features are a four compartmental representation of free leucine kinetics (compartments 1 to 4), a sequence of lipoprotein compartments accounting for the stepwise delipidation of $VLDL_1$ through $VLDL_2$ and IDL to LDL (compartments 6 to 7, 9 to 10, 12, 14) plus the three remnant compartments 8, 11 and 13 for $VLDL_1$, $VLDL_2$ and IDL particles that are removed directly from plasma. Free leucine and the apo B-containing compartments 6, 9, 12 and 14 in the $VLDL_1$, $VLDL_2$, IDL and LDL density ranges are linked via a delay element (compartment 5) that is set at 0.5 hours, accounting for the

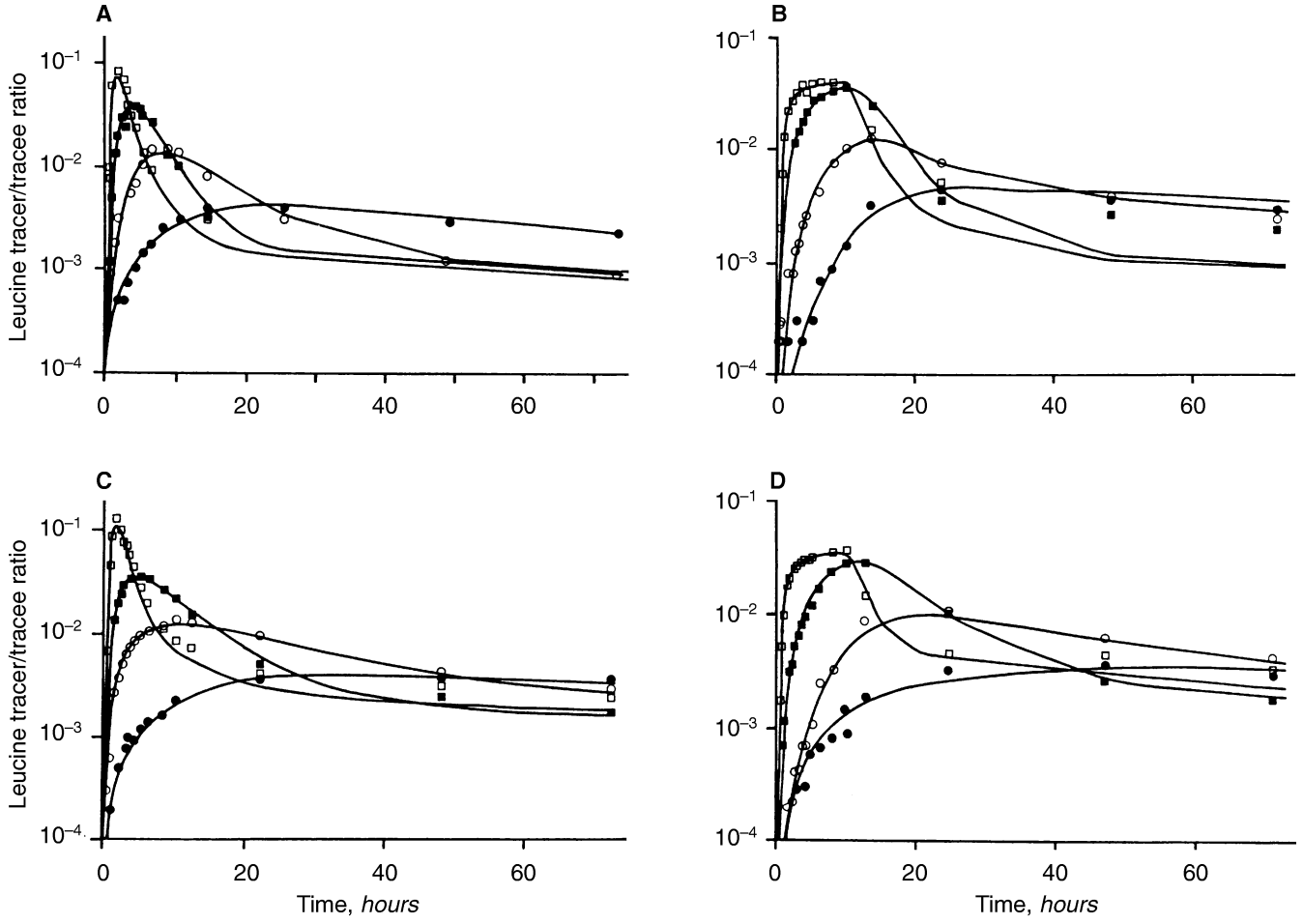


**Fig. 1. Time courses of leucine tracer/tracee ratios from $VLDL_1$ (□), $VLDL_2$ (■), IDL (○) and LDL (●) in two patients with nephrotic syndrome (*C*, KF, and *D*, JO) and two normal control subjects (*A*, MM, and *B*, WS).** The ordinate represents leucine tracer/tracee ratios as calculated from GC-MS measurements. Tracer was given as a bolus in patients MM and KF and as a primed constant infusion in WS and JO. Observed data are given as symbols and calculated values are represented by continuous lines.

time required for apo B biosynthesis. Compartment 15 allows for some intra-/extravascular exchange of LDL that is not observed for less dense lipoproteins. Requirements for *a priori* system identifiability in accordance with physiological considerations led to the introduction of the following model constraints [24]: $k_{2,1} = k_{1,2}$; $k_{8,6} = k_{11,9}$; $k_{0,8} = k_{0,11}$; $k_{7,6} = k_{10,9}$; $k_{13,10} = k_{0,13}$; $k_{0,10} = k_{0,12}$; $k_{14,15} = 2.5 * k_{15,14}$. Input of native leucine was represented in the model by $U_1$. To simulate the specific tracer enrichment in the immediate precursor pool for apo B synthesis (compartment 5) and to fit observed tracer/tracee curves for both plasma and lipoproteins, an additional tracee input ($U_5$) had to be allowed into compartment 5. $U_5$ is an additional unknown parameter that can be identified by multicompartmental model analysis [24].

The model devised for analysis of albumin metabolism is shown in Figure 2B. It consists of one plasma albumin compartment and a second extravascular compartment allowing for intra-/extravascular exchange. The plasma compartment is linked via a delay element to the same four compartmental model for free leucine that was used for apo B kinetic analysis (Fig. 2A). In nephrotic patients the calculated rate of albumin elimination from plasma (FER) comprises both urinary albumin excretion and albumin degradation by physiological mechanisms. The latter, which is expressed as fractional catabolic rate (FCR), was calculated from the difference between total albumin elimination and urinary albumin loss.

### Kinetic analysis by monoexponential curve fitting

In subjects who received the amino acid tracer as a primed constant infusion the fractional elimination rate of albumin (FER), was also determined by fitting a monoexponential term to the observed data for albumin tracer enrichment using the numerical mode of SAAM II. The following formula was applied [32]:

$$y(t) = B_{max} * [1 - e^{-FER * (t-0.5)}] \quad (3)$$

where y (t) is the tracer/tracee ratio for albumin at time t and $B_{max}$ is the plateau of the tracer/tracee ratio reached by

**Table 4.** Metabolic parameters of apolipoprotein B, a constituent of $VLDL_1$, $VLDL_2$, IDL and LDL from patients with nephrotic syndrome and from normal controls

| $VLDL_1$ (Sf 60–400) | Synthesis *mg/kg/day* | Pool *mg* | Transfer to $VLDL_2$ *pools/day* | Catabolism *pools/day* |
|---|---|---|---|---|
| Patients | | | | |
| WM | 19.9 | 574 | 2.02 | 1.10 |
| KF | 24.4 | 127 | 16.55 | 3.06 |
| JO | 9.8 | 70 | 8.55 | 1.16 |
| HA | 16.3 | 48 | 23.07 | 1.73 |
| MC | 25.0 | 109 | 6.62 | 10.80 |
| EA | 12.8 | 35 | 23.55 | 0.18 |
| ED | 14.5 | 125 | 4.49 | 2.03 |
| Median | 16.3 | 109 | 8.55 | 1.73 |
| Range | 9.8–25.0 | 35–574 | 2.0–23.6 | 0.2–10.8 |
| HS | 17.7 | 536 | 2.86 | 0.11 |
| KD | 26.8 | 464 | 3.38 | 1.99 |
| Controls | | | | |
| BG | 9.4 | 62 | 12.63 | 0.00 |
| CE | 19.4 | 53 | 8.66 | 13.77 |
| MM | 5.7 | 23 | 11.00 | 9.16 |
| BJ | 20.5 | 47 | 7.83 | 18.30 |
| RB | 25.4 | 114 | 10.94 | 12.77 |
| CS | 12.6 | 101 | 9.28 | 0.09 |
| WS | 17.1 | 59 | 10.43 | 13.86 |
| JD | 12.9 | 18 | 27.23 | 16.52 |
| GH | 18.7 | 24 | 24.50 | 20.41 |
| Median | 17.1 | 53 | 10.94 | 13.77 |
| Range | 5.7–25.4 | 18–114 | 7.8–27.2 | 0.0–20.4 |
| *P* value | | | | |
| Neph. vs. Cont. | 0.634 | 0.064 | 0.315 | 0.081 |

| IDL (Sf 12–20) | Synthesis *mg/kg/day* | Flux from $VLDL_2$ *mg/kg/day* | Pool *mg* | Transfer to LDL *pools/day* | Catabolism *pools/day* |
|---|---|---|---|---|---|
| Patients | | | | | |
| WM | 0.6 | 14.2 | 605 | 1.28 | 0.92 |
| KF | 3.0 | 15.8 | 991 | 1.12 | 0.81 |
| JO | 0.6 | 9.6 | 783 | 0.56 | 0.35 |
| HA | 4.3 | 16.7 | 2245 | 0.65 | 0.03 |
| MC | 1.6 | 11.2 | 860 | 0.99 | 0.14 |
| EA | 2.8 | 21.6 | 794 | 1.62 | 0.37 |
| ED | 2.3 | 10.5 | 537 | 0.80 | 0.53 |
| Median | 2.3 | 14.2 | 794 | 0.99 | 0.37 |
| Range | 0.6–4.3 | 9.6–21.6 | 537–2245 | 0.6–1.6 | 0.0–0.9 |
| HS | 1.1 | 13.5 | 556 | 1.28 | 1.09 |
| KD | 3.0 | 15.1 | 1008 | 0.89 | 0.78 |
| Controls | | | | | |
| BG | 0.5 | 10.6 | 337 | 2.15 | 0.59 |
| CE | 1.3 | 8.1 | 255 | 1.70 | 0.57 |
| MM | 6.9 | 4.7 | 534 | 1.74 | 0.02 |
| BJ | 1.0 | 6.6 | 328 | 1.40 | 0.00 |
| RB | 4.9 | 13.8 | 811 | 1.97 | 0.47 |
| CS | 2.3 | 9.8 | 209 | 2.42 | 1.93 |
| WS | 0.5 | 10.9 | 442 | 1.58 | 0.58 |
| JD | 2.4 | 7.1 | 289 | 1.96 | 0.03 |
| GH | 5.5 | 9.5 | 277 | 1.79 | 1.30 |
| Median | 2.3 | 9.5 | 328 | 1.79 | 0.57 |
| Range | 0.5–6.9 | 4.7–13.8 | 209–811 | 1.4–2.4 | 0.0–1.9 |
| *P* value | | | | | |
| Neph. vs. Cont. | 0.916 | 0.013 | 0.004 | 0.002 | 0.832 |

| $VLDL_2$ (Sf 20–60) | Synthesis *mg/kg/day* | Flux from $VLDL_1$ *mg/kg/day* | Pool *mg* | Transfer to IDL *pools/day* | Catabolism *pools/day* |
|---|---|---|---|---|---|
| Patients | | | | | |
| WM | 6.0 | 12.9 | 766 | 1.67 | 0.55 |
| KF | 1.7 | 20.6 | 689 | 2.34 | 0.96 |
| JO | 1.2 | 8.6 | 248 | 2.71 | 0.06 |
| HA | 1.6 | 15.2 | 460 | 2.65 | 0.01 |
| MC | 2.1 | 9.5 | 400 | 2.12 | 0.09 |
| EA | 9.0 | 12.8 | 211 | 6.65 | 0.05 |
| ED | 5.2 | 10.0 | 201 | 2.92 | 1.32 |
| Median | 2.1 | 12.8 | 400 | 2.65 | 0.09 |
| Range | 1.2–9.0 | 8.6–20.6 | 201–766 | 1.7–6.7 | 0.0–1.3 |
| HS | 6.0 | 17.0 | 691 | 1.76 | 1.23 |
| KD | 7.2 | 16.8 | 893 | 1.57 | 0.93 |
| Controls | | | | | |
| BG | 2.0 | 9.4 | 111 | 7.95 | 0.62 |
| CE | 1.0 | 7.5 | 103 | 4.80 | 0.21 |
| MM | 1.8 | 3.1 | 126 | 3.00 | 0.14 |
| BJ | 0.5 | 6.1 | 131 | 3.03 | 0.01 |
| RB | 2.1 | 11.7 | 233 | 6.28 | 0.00 |
| CS | 3.3 | 12.5 | 163 | 4.51 | 2.74 |
| WS | 3.6 | 7.4 | 115 | 7.87 | 0.01 |
| JD | 2.4 | 8.0 | 86 | 4.95 | 2.28 |
| GH | 4.3 | 10.2 | 60 | 9.07 | 4.70 |
| Median | 2.1 | 8.0 | 115 | 4.95 | 0.21 |
| Range | 0.5–4.3 | 3.1–12.5 | 61–233 | 3.0–9.1 | 0.0–4.7 |
| *P* value | | | | | |
| Neph. vs. Cont. | 0.525 | 0.023 | 0.002 | 0.007 | 0.711 |

| LDL(Sf 0–12) | Synthesis *mg/kg/day* | Flux from IDL *mg/kg/day* | Pool *mg* | Catabolism *pools/day* | Total Apo B production *mg/kg/day* |
|---|---|---|---|---|---|
| Patients | | | | | |
| WM | 0.0 | 8.6 | 2660 | 0.29 | 26.5 |
| KF | 1.5 | 10.9 | 4310 | 0.29 | 30.6 |
| JO | 3.3 | 6.3 | 3366 | 0.20 | 14.8 |
| HA | 7.8 | 19.9 | 4239 | 0.48 | 30.0 |
| MC | 1.9 | 11.2 | 3090 | 0.32 | 30.6 |
| EA | 8.3 | 19.8 | 4339 | 0.41 | 32.9 |
| ED | 0.9 | 7.7 | 2282 | 0.21 | 22.9 |
| Median | 1.9 | 10.9 | 3368 | 0.29 | 30.0 |
| Range | 0.0–8.3 | 6.3–19.9 | 2282–4439 | 0.2–0.5 | 14.8–32.9 |
| HS | 1.2 | 7.9 | 2288 | 0.36 | 26.0 |
| KD | 0.2 | 9.6 | 3347 | 0.27 | 37.0 |
| Controls | | | | | |
| BG | 1.9 | 8.7 | 1845 | 0.48 | 13.8 |
| CE | 0.5 | 7.1 | 1559 | 0.30 | 22.3 |
| MM | 3.8 | 11.5 | 1962 | 0.63 | 18.2 |
| BJ | 4.6 | 7.7 | 1730 | 0.42 | 26.6 |
| RB | 1.9 | 15.1 | 2756 | 0.65 | 34.2 |
| CS | 3.4 | 6.7 | 1337 | 0.57 | 21.6 |
| WS | 0.0 | 8.4 | 2577 | 0.27 | 21.3 |
| JD | 4.3 | 9.4 | 1649 | 0.50 | 22.0 |
| GH | 0.2 | 8.7 | 1306 | 0.39 | 28.6 |
| Median | 1.9 | 8.7 | 1730 | 0.48 | 22.0 |
| Range | 0.0–4.6 | 6.7–15.1 | 1306–2756 | 0.3–0.7 | 13.8–34.2 |
| *P* value | | | | | |
| Neph. vs. Cont. | 0.832 | 0.597 | 0.003 | 0.034 | 0.153 |

$VLDL_1$ 10 hours after continuous tracer infusion. Since $VLDL_1$ is a rapidly turning over hepatic protein its $B_{max}$ value can be taken as a measure of the tracer enrichment of the hepatic precursor pool of protein synthesis [33].

## Statistical analysis

Statistical analysis was performed using Minitab software, version 11 (Minitab Inc., State College, PA, USA).

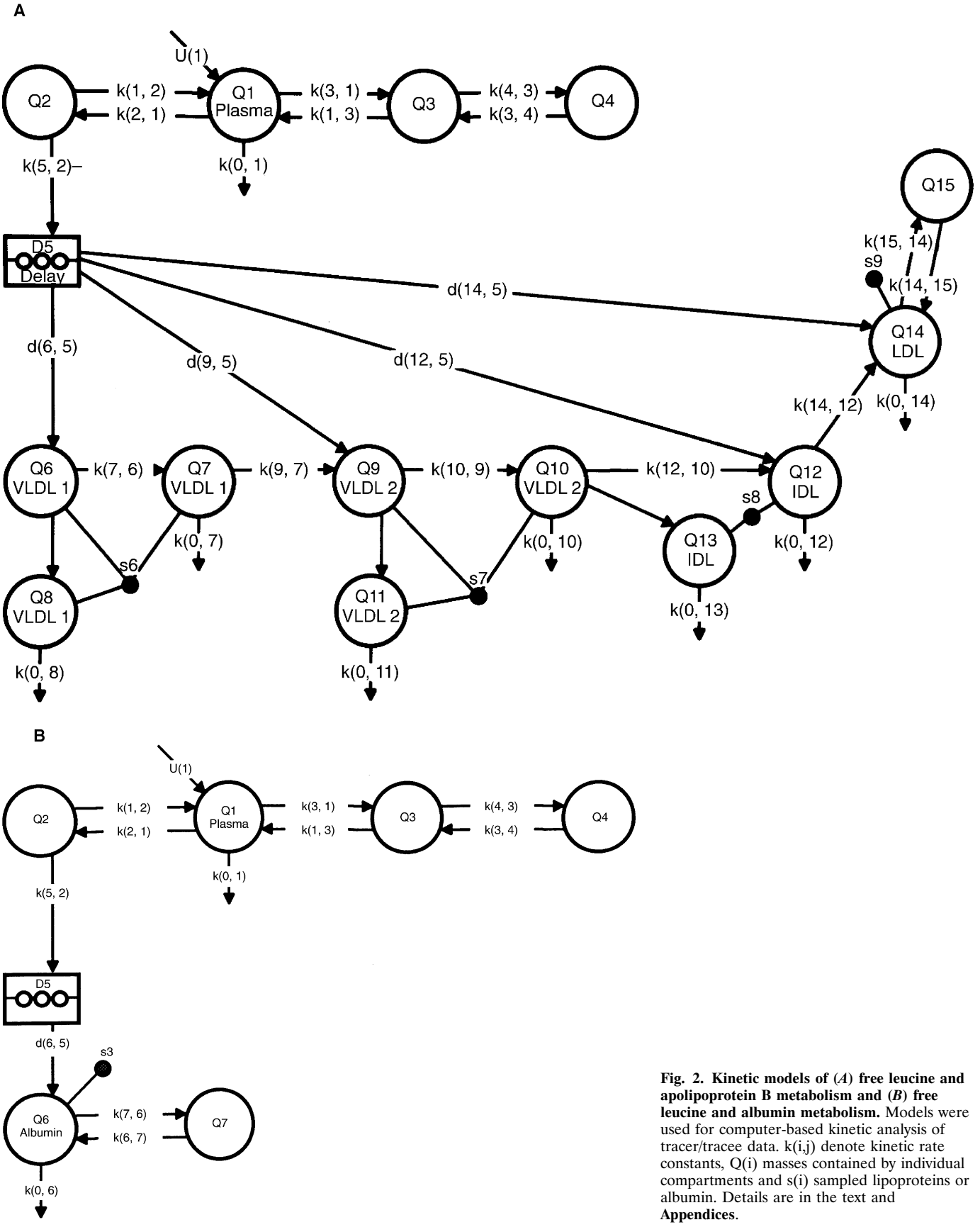


**Fig. 2. Kinetic models of (*A*) free leucine and apolipoprotein B metabolism and (*B*) free leucine and albumin metabolism.** Models were used for computer-based kinetic analysis of tracer/tracee data. k(i,j) denote kinetic rate constants, Q(i) masses contained by individual compartments and s(i) sampled lipoproteins or albumin. Details are in the text and **Appendices**.

Results are shown as means and SDS or as medians and ranges. Differences between nephrotic patients and controls were evaluated by a non-parametric test (Kruskal-Wallis). Correlations between metabolic parameters within study groups were examined by Pearson's correlation matrix and regression analysis. For variables which were not normally distributed as demonstrated by Minitab normality plots the Spearman's rank correlation coefficient was determined prior to regression analysis. Differences and correlations with $P < 0.05$ were considered as statistically significant.

## RESULTS

Seven patients, five males and two females, suffering from nephrotic syndrome due to primary renal disease participated in this study. Two subjects (HS and KD) who had nephrosis as well as moderate renal failure were also studied, but their results were considered separately and are reported at the end of this section. Kidney biopsies revealed membranous glomerulonephritis in five and focal segmental glomerulosclerosis in three subjects (Table 1). Proteinuria was in the range of 7.3 to 16.3 g/24 hours, and about 85% of urinary protein was determined to be albumin. Plasma albumin levels were significantly reduced (2.6 ± 0.4 vs. 4.9 ± 0.4 g/dl in controls). None of these patients presented with signs of chronic renal failure such as azotemia, hypertension or renal anemia. Renal disease secondary to hypertension or diabetes was excluded in all subjects. Serum creatinine concentrations were in the range of 0.4 to 1.2 mg/dl. All patients displayed substantial hyperlipidemia with total cholesterol concentrations of 259 to 500 mg/dl and triglycerides of 105 to 440 mg/dl. When apolipoprotein B-containing lipoproteins were prepared and analyzed separately concentrations of $VLDL_2$, IDL and LDL were two- to threefold higher in patients compared to controls (Table 4). $VLDL_1$ concentrations also showed a trend towards higher levels. In contrast, HDL concentrations were similar and lipid and protein compositions of $VLDL_1$, $VLDL_2$, IDL and LDL were the same in patients and in controls (data not shown). Patients and controls were on standard diets with a protein content estimated from questionnaires of 15 to 25% of the daily calorie intake. Seven-day weighed food intake protocols from a subset of individuals (3 patients and 4 controls) indicated no differences in protein consumption, which was in the range of 0.7 to 1.6 g/kg/day.

Seven males and two females served as a control group. Subjects were normolipidemic with total cholesterol levels of 155 to 232 mg/dl and triglyceride levels of 40 to 122 mg/dl. Patients and controls were of similar body frame (body mass index 26 ± 4 vs. 23 ± 4 $kg/m^2$). On average, patients were nine years older than controls (44 ± 15 vs. 35 ± 8 years; Tables 2 and 3).

The typical curve patterns for tracer/tracee ratios as measured in the $VLDL_1$, $VLDL_2$, IDL and LDL fractions are shown in Figure 1 for two patients and for two control subjects who received the tracer amino acid either as a bolus injection or as an infusion. Multicompartmental kinetic analysis was employed to determine rates of apolipoprotein B production, transfer and catabolism for each of the four lipoproteins under investigation. Calculations were based on observed tracer/tracee ratios, measured apo B pools and the metabolic model shown in Figure 2A. Individual values for each study participant are given in Table 4. The rate of direct $VLDL_1$ catabolism and of $VLDL_1$ to $VLDL_2$ transfer were lower in patients than in controls, in line with the nonsignificant increase in plasma $VLDL_1$ observed in these patients. A more than threefold increase of $VLDL_2$ concentrations (13.2 vs. 3.9 mg/dl, $P = 0.001$) was mainly due to a delayed transfer of $VLDL_2$ to IDL (2.65 vs. 4.95 pools/day, $P = 0.007$). The absolute amount of $VLDL_2$ transferred into IDL, calculated as the product of the fractional transfer rate and the size of the $VLDL_2$ pool, was higher in patients than in controls (14.2 vs. 9.5 mg/kg/day, $P = 0.013$). This is because the substantially increased $VLDL_2$ pool more than compensated for the significant reduction of the $VLDL_2$ transfer rate. The higher influx from $VLDL_2$ in combination with a diminished rate of IDL to LDL transfer (0.99 vs. 1.79 pools/day, $P = 0.002$) gave rise to a twofold increase of the IDL concentration (28.0 vs. 12.2 mg/dl, $P = 0.001$). Most of the IDL mass (71 ± 16% of the total) was attributed to a compartment (comp. 13 in Fig. 2) that does not contribute to the formation of LDL but is cleared by direct catabolism of particles. The remainder of IDL represented by compartment 12 was further delipidated to become eventually LDL (comp. 14). In controls the IDL mass was more evenly distributed between the two IDL compartments (48% and 52%, respectively). The LDL concentration was more than 50% higher in patients than in controls (105.6 vs. 63.9 mg/dl, $P = 0.001$) due to a significantly lower LDL fractional catabolic rate (0.29 vs. 0.48 pools/day, $P = 0.034$). The rate of total apolipoprotein B production was higher in patients than in controls (30.0 vs. 22.0 mg/kg/day), but the difference failed to reach significance ($P = 0.153$). The distribution of direct apo B secretion into different lipoprotein fractions was similar in both groups: on average 60 to 65% of the total apo B production was observed in the $VLDL_1$ density range, 10 to 15% occurred as a constituent of $VLDL_2$, and the remaining 20 to 30% of direct apo B production were distributed between IDL and LDL.

The time courses for albumin tracer/tracee ratios from two patients with nephrotic syndrome and from two control subjects are given in Figure 3. Based on multicompartmental kinetic analysis and the model of albumin metabolism shown in Figure 2 b. rates of albumin synthesis and elimination from plasma were calculated as listed in Table 5 and 6. The fractional rate of albumin elimination (FER) was almost three times higher in patients than the fractional rate of albumin catabolism (FCR) in normal controls

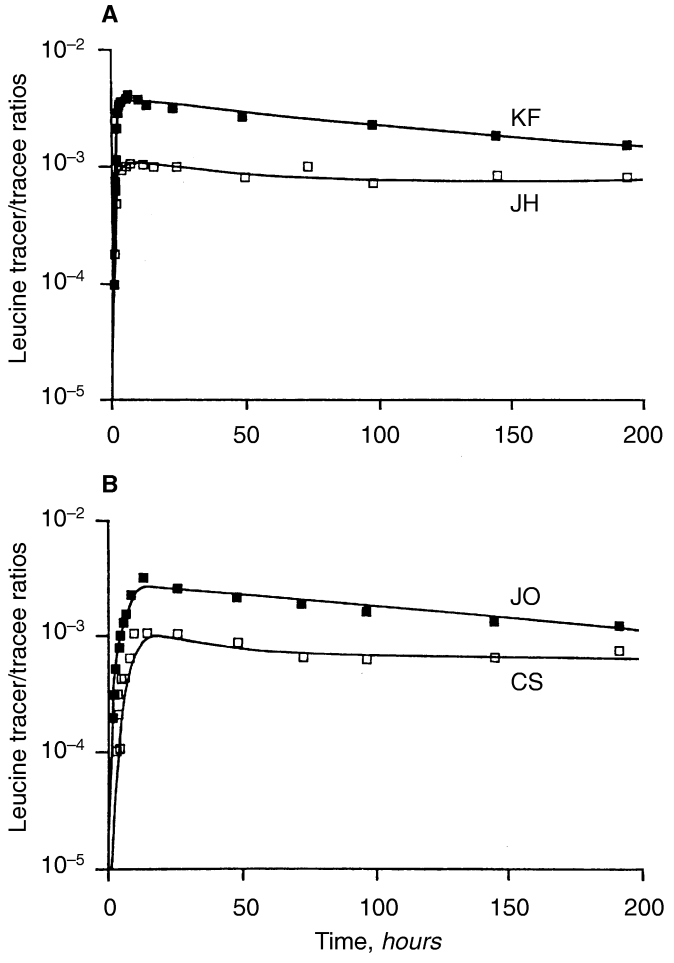


**Fig. 3. Time courses of leucine tracer/tracee ratios from albumin in two patients with nephrotic syndrome (■ KF in A and ■ JO in B) and in two normal control subjects (□ JH in A and □ CS in B).** Tracer was given as a bolus in JH and KF (*A*) and as a primed constant infusion in CS and JO (*B*). Observed data are given as symbols, calculated values are represented by continuous solid lines.

(median 0.196 vs. 0.067 pools/day, $P = 0.003$), which was due to marked urinary protein losses in nephrosis (Table 1). In nephrotics the fractional catabolic rate (FCR) of albumin due to endogenous degradation, calculated as the difference between albumin eliminated from plasma and renal albumin loss, was very similar to the albumin FCR measured for controls, indicating that physiological mechanisms of albumin degradation were unperturbed in this group of patients. The absolute amount of albumin catabolized per day, however, was decreased by 40% in patients compared to controls (5.36 vs. 9.12 g/day, $P = 0.010$). Albumin synthesis was determined in the range of 148 to 220 mg/kg/day in nephrotics, which is on average 45% higher than the values found in normal controls (96 to 152 mg/kg/day, $P = 0.034$). This increase in albumin production was not sufficient to compensate for renal albumin excretion with the result of an almost 50% reduction of the albumin serum concentration (2.6 ± 0.4 vs. 4.9 ± 0.4 g/dl, $P = 0.003$). Where the tracer amino acid had been administered as a primed constant infusion the fractional elimination rate (FER) of albumin was also determined by an alternative method whereby a monoexponential function was fitted to the albumin tracer/tracee curve observed during the time of infusion. Values for albumin FER calculated in five subjects either by multicompartmental modeling or by monoexponential curve fitting were virtually identical ($r = 0.93$).

Correlations between the metabolism of albumin and of apolipoprotein B containing lipoproteins were examined by linear regression analysis within the group of nephrotic patients. Neither plasma albumin concentrations nor rates of albumin synthesis were correlated to the rate of total apolipoprotein B production ($P = 0.37$ and $P = 0.86$, respectively; Fig. 4). There was also no significant correlation between these parameters and any of the other individual production rates of apo B in the $VLDL_1$, $VLDL_2$, IDL or LDL density range. However, an inverse relationship between the rate of $VLDL_2$ synthesis and the plasma concentration of albumin ($r = -0.74$, $P = 0.034$) could be demonstrated.

Two patients (HS, KD) with nephrotic syndrome and moderate azotemia were studied in addition to the group of seven patients with normal excretory renal function. These subjects showed a relatively low level of proteinuria and almost normal albumin plasma concentrations. Their VLDL concentrations were markedly increased but their LDL levels were in the near normal range. Kinetic analysis of their apo B metabolism revealed high rates of $VLDL_1$- and $VLDL_2$-apo B production and low rates of $VLDL_1$ and $VLDL_2$ transfer. Otherwise, their albumin and apolipoprotein B metabolism was similar to that observed in the group of seven other patients. The lack of correlation between rates of albumin and apo B synthesis mentioned above was independent of whether or not the two patients were included in the correlation studies.

## DISCUSSION

Reduced plasma albumin concentrations and increased levels of LDL and other apo B-containing lipoproteins are typical findings in patients with the nephrotic syndrome. The present study was designed to investigate to what extent urinary protein loss and consecutive changes in albumin metabolism contribute to the abnormalities in apolipoprotein B metabolism observed in nephrosis. To this end we studied the metabolism of apo B-containing lipoproteins and of albumin in nine nephrotic patients using a novel approach based on stable isotope labeled amino acid tracer kinetics and simultaneous multicompartmental model analysis. Since the tracer is incorporated into both newly synthesized apo B and albumin alike, their metabolism can be studied under identical circumstances in the same individual at the same time. This avoids some of

**Table 5.** Albumin metabolic parameters in patients with nephrotic syndrome

| Name | Alb. synthesis *mg/kg/day* | Alb. pool *g* | Alb. FER *pools/day* | abs. Alb. elim. | Albuminuria | abs. Alb. catab | Alb. FCR *pools/day* |
|---|---|---|---|---|---|---|---|
| | | | | *g/day* | | | |
| WM | 219 | 98.4 | 0.200 | 19.67 | 14.0 | 5.67 | 0.058 |
| KF | 218 | 102.5 | 0.217 | 22.24 | 14.0 | 8.24 | 0.080 |
| JO | 169 | 77.9 | 0.152 | 11.84 | 10.1 | 1.74 | 0.022 |
| HA | 220 | 82.4 | 0.196 | 16.15 | 9.6 | 6.55 | 0.080 |
| MC | 149 | 80.7 | 0.140 | 11.30 | 9.3 | 2.00 | 0.024 |
| EA | 148 | 42.2 | 0.226 | 9.53 | — | — | — |
| ED | 173 | 55.8 | 0.173 | 9.66 | 4.6 | 5.06 | 0.091 |
| Median | 173 | 80.7 | 0.196 | 11.84 | 9.9 | 5.37 | 0.069 |
| Range | 148–220 | 42.2–102.5 | 0.14–0.23 | 9.5–22.2 | 4.6–14.0 | 1.7–8.2 | 0.02–0.09 |
| HS | 185 | 138.90 | 0.120 | 16.67 | 5.4 | 11.27 | 0.081 |
| KD | 231 | 175.83 | 0.122 | 21.45 | 3.9 | 17.55 | 0.100 |
| *P* value | | | | | | | |
| Neph. vs. Cont. | 0.010 | 0.007 | 0.003 | | | 0.010 | |

Abbreviations are: Alb., albumin; FER, rate of elimination of albumin from plasma; FCR, fractional catabolism rate; catab, catabolism; abs., absolute.

**Table 6.** Albumin metabolic parameters in normal controls

| Name | Alb. synthesis *mg/kg/day* | Alb. pool *g* | Alb. FCR *pools/day* | abs. Alb. catab. *g/day* |
|---|---|---|---|---|
| CE | 153 | 115.1 | 0.081 | 9.33 |
| RB | 118 | 187.0 | 0.067 | 12.53 |
| CS | 130 | 145.2 | 0.067 | 9.73 |
| WS | 96 | 159.2 | 0.050 | 7.96 |
| JD | 149 | 97.9 | 0.091 | 8.91 |
| GH | 124 | 117.4 | 0.060 | 7.04 |
| Median | 127 | 131.3 | 0.067 | 9.12 |
| Range | 96–153 | 97.9–187.0 | 0.05–0.09 | 7.04–12.53 |

Abbreviations are as in Table 5.

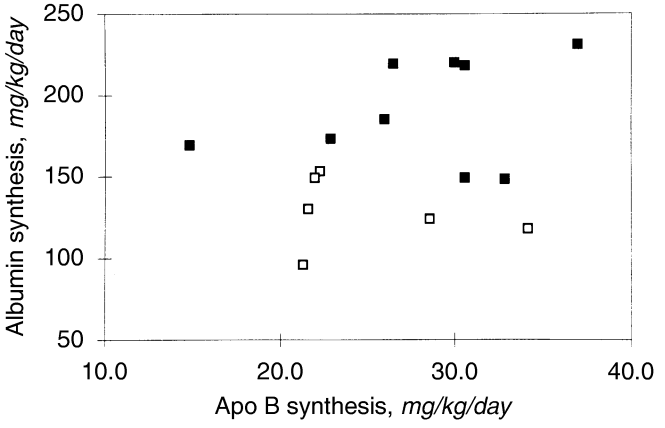


**Fig. 4. Lack of association of the rate of albumin synthesis and the rate of total apolipoprotein B production in nine patients with nephrotic syndrome (□) and in six control subjects (■).**

the ambiguities of earlier work addressing the interconnections between albumin and lipoprotein metabolism in nephrotic patients. Seven out of nine patients participating in this study were similar in that they were markedly hypoalbuminemic and hypercholesterolemic but showed no signs of chronic renal failure, which in itself may cause secondary hyperlipidemia [2]. These patients were compared to nine normolipidemic subjects in the control group. Additionally, two nephrotic subjects (HS and KD) with concomitant mild to moderate renal failure and hypertriglyceridemia were examined. Results from their metabolic studies were of particular interest in comparison to previously published work where often nephrotic patients with some degree of renal failure have been investigated [8–10].

Increased concentrations of $VLDL_2$, IDL and LDL in the patient group were the result of three metabolic abnormalities that were: (*a*) significantly reduced rates of $VLDL_2$ to IDL and of IDL to LDL transfer, (*b*) a significantly lower LDL-FCR, and (*c*) a trend for an increased rate of total apolipoprotein B production. In a previous study we investigated the metabolism of apo B containing lipoproteins in eight nephrotic patients with concomitant mild to moderate renal failure using a dual radioiodinated VLDL tracer technique [8]. Similar deviations from normal apo B metabolism were observed: (*a*) the rates of transfer from $VLDL_1$ to $VLDL_2$ and from $VLDL_2$ to IDL were significantly reduced, (*b*) LDL-FCRs were in most patients lower than in controls, and (*c*) apo B production in particular in the $VLDL_2$ density range was increased.

Delipidation of lipoproteins from triglyceride rich $VLDL_1$ down to LDL is mediated by two lipolytic enzymes, lipoprotein lipase and hepatic lipase, which have partially overlapping substrate specificities [34, 35]. Catalytic activities of both enzymes have been studied in human and in experimental nephrosis and were found to be significantly reduced [13, 36, 37], although these observations were not consistently confirmed by others [38, 39]. Possible mechanisms for impaired lipolytic activity are the urinary loss of a low molecular weight glycoaminoglycan, which has been shown to act as an activator of lipoprotein lipase *in vivo* and *in vitro* [16], or a relative depletion of apo CII, an obligatory activator of lipoprotein lipase, which in nephrotic patients was found to be reduced relative to the increased number of VLDL particles [36]. Proteinuria in our patients was positively correlated with the concentration of $VLDL_2$ ($r$ =

+ 0.76, $P = 0.077$) and negatively with the transfer rate from $VLDL_2$ to IDL ($r = -0.82$, $P = 0.047$), which is in support of the concept that the urinary loss of an activator of lipolytic enzymes is of importance. Since in *in vitro* experiments VLDL isolated from nephrotic subjects is insufficiently hydrolyzed when incubated with normal lipoprotein lipase [40], it is assumed that both substrate and enzyme abnormalities play a role in delayed lipoprotein delipidation in nephrotic syndrome. In the present study $VLDL_1$ delipidation was only marginally decreased but the delipidation of $VLDL_2$ and IDL was significantly reduced. It is likely, therefore, that hepatic lipase with its preferential affinity for $VLDL_2$ and IDL was primarily affected in this group of patients. However, patients HSC and KDH, who were more akin to the subjects from our previous study, showed rather low rates of $VLDL_1$ delipidation, which is in accordance with the significant reduction in this delipidation step reported in this study [8].

The catabolism of LDL has been studied in patients with nephrotic syndrome by several groups using different study protocols. LDL turnovers with an exogenously radioiodinated LDL tracer [6, 9, 10], the dual radioiodinated VLDL tracer protocol [8] and endogenous labeling with stable isotopes [12] as used in the present study have all been employed. In four studies of nephrotic patients with hypercholesterolemia or mixed hyperlipidemia the LDL-FCR was found to be in the lower range of normal [6, 8, 10] or significantly reduced [9]. This is in accordance with the present study where the LDL-FCR in patients was on average 35% lower than in controls. We have shown previously that the delay in LDL catabolism observed in nephrotic subjects was mainly due to a reduced clearance via the LDL receptor pathway [9]. Down-regulation of hepatic LDL receptors might be caused by increased hepatic cholesterogenesis that was observed at least in experimental nephrosis in rats as a result of increased retention of mevalonate [15]. However, since mevalonate levels were not determined in the present study this mechanism remains speculative. Alternatively, LDL from hypertriglyceridemic subjects has been shown *in vitro* to bind less avidly to LDL receptors, which also explains why LDL in nephrotic subjects is catabolized more slowly. Indeed, in a recent study of normolipidemic to moderately hyperlipidemic subjects we were able to demonstrate that the LDL-FCR in the upper triglyceride tertile (>140 mg/dl) was 50% lower than in the lower tertile (<80 mg/dl). It should be noted that in two studies examining nephrotic patients with excessive hypertriglyceridemia (>500 mg/dl) the LDL-FCR was actually found to be higher than in normolipidemic controls [10, 12]. This is in line with similar findings in comparable states of extreme hypertriglyceridemia due to other metabolic disorders [36] and reflects an increased LDL degradation by LDL receptor-independent mechanisms [41].

A trend towards an increased production of apolipoprotein B was the third component, after impaired lipoprotein delipidation and decreased LDL catabolism, which contributed to the hyperlipidemia observed in the patient group. This difference, although statistically not significant, is in line with similar findings reported from other studies in humans [8–12] and in experimental animals [13, 14]. In nephrotic syndrome both increased triglyceride production and increased cholesterogenesis have been observed in animal studies [15, 42] and in humans [43]. Evidence from *in vitro* experiments with hepatoma cell lines suggests that intracellular availability of lipids is an important regulator of hepatic VLDL secretion [44]. *In vivo* studies in normolipidemic and hyperlipidemic subjects support the view that increased biosynthesis of triglycerides [45] and of cholesterol [46] are associated with increased production rates for large triglyceride rich $VLDL_1$ or smaller cholesteryl ester rich $VLDL_2$. It is likely that both mechanisms contribute to the overproduction of apo B-containing lipoproteins observed in nephrotic patients.

To find out whether this increase in apo B production was linked to changes in albumin metabolism, in particular to an increased rate of albumin synthesis, we took advantage of the possibility offered by a stable isotope labeled amino acid tracer study to simultaneously examine the synthesis and degradation of more than one protein [26]. The fractional rate of albumin elimination from plasma was almost three times increased and showed the expected positive correlation with the rate of urinary protein loss ($r = +0.71$, $P = 0.03$). Albumin synthesis was significantly higher in patients compared to controls, a finding confirmed by earlier studies in animals and in humans [47, 48]. In a recent study by Ballmer and Weber, albumin synthesis was determined in seven nephrotic patients and eight controls by use of stable isotope labeled leucine and the "flooding dose protocol" [22]. Their absolute values were only slightly higher (214 ± 48 and 144 ± 10 mg/kg/day in patients and controls, respectively) and the relative increase of 48% in patients was almost identical to our study. In a study by Kaysen et al rates of albumin synthesis were measured using radioiodinated albumin and a pharmacokinetic method [23]. In 13 nephrotic patients with marked proteinuria, the rate of albumin production was either within the normal range or significantly increased depending on the protein content of the diet. Quantitative dietary records were taken only in three of our patients, but it should be noted that the protein content of their diets (0.7 to 1.6 g/kg/day) varied within the same range as in the study above.

In nephrotic syndrome, the causal relationships between disturbances in the metabolism of albumin on the one hand and apo B-containing lipoproteins on the other hand are not clear. One hypothesis suggested by early workers in the field is that a general increase in hepatic protein synthesis is responsible for both overproduction of lipoproteins and albumin [21]. Linear regression analysis of the production

rates for albumin and apolipoprotein B that were simultaneously determined in this study gave no evidence of a positive correlation. This negative result is supported by the absence of a correlation between albumin synthesis and serum cholesterol concentrations reported by Kaysen et al in the study mentioned above [23]. In contrast to our study, a positive correlation was reported between albumin synthesis and triglyceride concentrations, but this was much weaker than the positive association with the rate of renal albumin clearance. A similar association between the $VLDL_2$ concentration and the rate of proteinuria observed in our patient group has been mentioned before.

A second mechanism proposed for the increased hepatic production of apo B-containing lipoproteins in nephrotic subjects is related to reduced albumin concentrations in plasma rather than the increase in hepatic albumin synthesis *per se*. The inverse correlation between albumin concentrations and the rate of $VLDL_2$ production observed in this study is compatible with this concept. Hypoalbuminemia is associated with a reduction in the plasma oncotic pressure, and it has been suggested that this rather than a direct effect of albumin may result in increased hepatic protein production [18, 49]. Early studies have shown that the hyperlipidemia in nephrotic patients can be reversed by administration of either albumin or dextran infusions [49]. More recently, *in vitro* studies with Hep G2 cells demonstrated a dose-dependent inhibitory effect of albumin on cellular apo B production [19, 20] and a suppression of intracellular apo B degradation at low albumin levels [50]. It has also been shown in a rat hepatoma cell line that apo B and albumin gene transcription are regulated by the oncotic pressure in the culture medium [18]. The inverse association between $VLDL_2$ synthesis and albumin concentration together with the significant increase in $VLDL_2$ production reported in our previous study [8] point at a mechanism that is either driven by a reduction in the plasma oncotic pressure or, as mentioned before, by an increase of cholesterol biosynthesis [42, 43]. Higher rates of triglyceride production, on the other hand, would primarily enhance the hepatic secretion of triglyceride rich large $VLDL_1$ rather than of cholesteryl ester rich smaller $VLDL_2$. However, in the absence of direct measurements the correlations within a small group of patients are too weak to support further speculation.

In conclusion, the present study shows that the predominant defects in apolipoprotein B metabolism in nephrotic patients are reduced rates of lipoprotein delipidation and catabolism combined with a less consistent increase in apo B production. The turnover of albumin is increased due to a threefold increase in the fractional rate of albumin elimination and a significant increase in albumin synthesis. Associations between metabolic parameters for apolipoprotein B and albumin are weak and in particular they were undetectable at the level of hepatic protein synthesis. Key features in nephrotic syndrome, impaired albumin and lipoprotein metabolism, seem to develop in parallel, probably precipitated through proteinuria, and direct interactions between the metabolism of both are of minor importance.

## ACKNOWLEDGMENTS

This work was supported by grants from the Deutsche Forschungsgemeinschaft (De 359/2) and the British-German Academic Research Collaboration Program (DAAD/ARC-313). Part of this work constitutes the medical thesis (Dr. med.) of C. Mathes.

*Reprint requests to PD Dr. med. Thomas Demant, Ph.D., Institut für Klinische Chemie, Klinikum Grosshadern, Ludwig-Maximilians-Universität, Marchioninistr. 15, D-81366 München, Germany.*
*E-mail: thomas.demant@klch.med.uni-muenchen.de*

## REFERENCES

1. Baxter JH, Goodman HC, Havel RJ: Serum lipid and lipoprotein alterations in nephrosis. *J Clin Invest* 39:455–465, 1960
2. Attman PO, Samuelsson O, Alaupovic P: Lipoprotein metabolism and renal failure. *Am J Kidney Dis* 21:573–592, 1993
3. Warwick GL, Packard CJ: Pathogenesis of lipid abnormalities in patients with nephrotic syndrome/proteinuria: Clinical implications. *Miner Electrolyte Metab* 19:115–126, 1993
4. Curry RC, Roberts WC: Status of the coronary arteries in the nephrotic syndrome. Analysis of 20 necropsy patients aged 15 to 35 years to determine if coronary atherosclerosis is accelerated. *Am J Med* 63:183–192, 1977
5. Ordonez JD, Hiatt RA, Killebrew EJ, Fireman BH: The increased risk of coronary heart disease associated with nephrotic syndrome. *Kidney Int* 44:638–642, 1993
6. Joven J, Villabona C, Vilella E, Masana L, Alberti R, Valles M: Abnormalities of lipoprotein metabolism in patients with the nephrotic syndrome. *N Engl J Med* 323:579–584, 1990
7. Appel GB, Blum CB, Chien S, Kunis CL, Appel AS: The hyperlipidemia of the nephrotic syndrome: Relation to plasma albumin concentration, oncotic pressure and viscosity. *N Engl J Med* 312:1544–1548, 1985
8. Warwick GL, Packard CJ, Demant T, Bedford D, Boulton-Jones JM, Shepherd J: Metabolism of apolipoprotein B-containing lipoproteins in subjects with nephrotic-range proteinuria. *Kidney Int* 40:129–138, 1991
9. Warwick GL, Caslake MJ, Boulton-Jones JM, Dagen M, Packard CJ, Shepherd J: Low-density lipoprotein metabolism in the nephrotic syndrome. *Metabolism* 39:187–192, 1990
10. Vega GL, Toto RD, Grundy SM: Metabolism of low density lipoprotein in nephrotic dyslipidemia: Comparison of hypercholesterolemia alone and combined hyperlipidemia. *Kidney Int* 47:579–586, 1995
11. Vega GL, Grundy SM: Lovastatin therapy in nephrotic hyperlipidemia: Effects on lipoprotein metabolism. *Kidney Int* 33:1160–1168, 1988
12. Aguilar-Salinas CA, Barrett PHR, Kelber J, Delmez J, Schonfeld G: Physiologic mechanisms of action of lovastatin in nephrotic syndrome. *J Lipid Res* 36:188–199, 1995
13. Garber DW, Gottlieb BA, Marsh JB, Sparks CE: Catabolism of very low density lipoproteins in experimental nephrosis. *J Clin Invest* 74:1375–1383, 1984
14. Marsh JB, Drabkin DL: Experimental reconstruction of metabolic pattern of lipid nephrosis: Key role of hepatic protein synthesis in hyperlipidemia. *Metabolism* 9:946–955, 1960
15. Golper TA, Feingold KR, Fulford MH, Siperstein MD: The role of circulating mevalonate in nephrotic hypercholesterolemia in the rat. *J Lipid Res* 27:1044–1051, 1986
16. Staprans I, Felts JM, Couser WG: Glycosaminoglycans and chylomicron metabolism in control and nephrotic rats. *Metabolism* 36:496–501, 1987
17. Yoshino G, Hirano T, Nagata K, Maeda E, Naka Y, Murata Y, Kazumi T, Kasuga M: Hypertriglyceridemia in nephrotic rats is due to a clearance defect of plasma triglyceride: Overproduction of triglyceride-rich lipoprotein is not an obligatory factor. *J Lipid Res* 34:875–884, 1993

18. Yamauchi A, Fukuhara Y, Yamamoto S, Yano F, Takenaka M, Imai E, Noguchi T, Tanaka T, Kamada T, Ueda N: Oncotic pressure regulates gene transcriptions of albumin and apolipoprotein B in cultured rat hepatoma cells. *Am J Physiol* 263:C397–C404, 1992
19. Cianflone K, Vu H, Zhang Z, Sniderman AD: Effects of albumin on lipid synthesis, apo B-100 secretion, and LDL catabolism in HepG2 cells. *Atherosclerosis* 107:125–135, 1994
20. Pullinger CR, North JD, Teng BB, Rifici VA, de Brito AER, Scott J: The apolipoprotein B gene is constitutively expressed in HepG2 cells: Regulation of secretion by oleic acid, albumin and insulin and measurement of the mRNA half-life. *J Lipid Res* 30:1065–1077, 1989
21. Marsh JB: Lipoprotein metabolism in experimental nephrosis. *J Lipid Res* 25:1619–1622, 1984
22. Ballmer PE, Weber BK, Roy-Chaudhury P, McNurlan MA, Watson H, Power DA, Garlick PJ: Elevation of albumin synthesis rates in nephrotic patients measured with $^{13}C$ leucine. *Kidney Int* 41:132–138, 1992
23. Kaysen GA, Gambertoglio J, Felts J, Hutchison FN: Albumin synthesis, albuminuria and hyperlipemia in nephrotic patients. *Kidney Int* 31:1368–1376, 1987
24. Demant T, Packard CJ, Demmelmair H, Stewart P, Bedynek A, Seidel D, Shepherd J: Sensitive methods to study human apolipoprotein B metabolism using stable isotope labelled amino acids. *Am J Physiol* 270:E1022–E1036, 1996
25. Parhofer KG, Barrett PHR, Bier D, Schonfeld G: Determination of kinetic parameters of apolipoprotein B metabolism using amino acids labeled with stable isotopes. *J Lipid Res* 32:1311–1323, 1991
26. Demant T, Mathes C, Bedynek A, Bosch T, Seidel D: Simultanous measurement of apolipoprotein B and albumin metabolism using gas chromatography-mass spectrometry and multicompartmental data analysis. *Eur J Clin Invest* 33:A26, 1995
27. Seman LJ, Jenner JL, McNamara JR, Schaefer EJ: Quantification of lipoprotein(a) in plasma by assaying cholesterol in lectin-bound plasma fraction. *Clin Chem* 40:400–403, 1994
28. Travis J, Pannell R: Selective removal of albumin from plasma. *Clin Chim Acta* 49:49–52, 1973
29. Peters T: Serum albumin. *Adv Prot Chem* 37:161–245, 1985
30. Adams RF: Determination of amino acid profiles in biological samples by gas chromatography. *J Chromatogr* 95:189–212, 1974
31. Cobelli C, Toffolo G, Foster D: Tracer-to-tracee ratio for analysis of stable isotope tracer data: Link with radioactive kinetic formalism. *Am J Physiol* 262:E968–E975, 1992
32. Foster DM, Barrett PHR, Toffolo G, Beltz WF, Cobelli C: Estimating the fractional synthetic rate of plasma apolipoproteins and lipids from stable isotope data. *J Lipid Res* 34:2193–2205, 1993
33. Reeds PJ, Hachey DL, Patterson BW, Motil KJ, Klein PD: VLDL apolipoprotein B-100, a potential indicator of the isotopic labeling of the hepatic protein synthetic precursor pool in humans: Studies with multiple stable isotopically labeled amino acids. *J Nutr* 122:457–466, 1991
34. Demant T, Gaw A, Watts GF, Durrington P, Buckley B, Imrie CW, Wilson C, Packard CJ, Shepherd J: Metabolism of apo B-100 containing lipoproteins in familial hyperchylomicronemia. *J Lipid Res* 34:147–156, 1993
35. Demant T, Carlson LA, Holmquist L, Karpe F, Nilsson-Ehle P, Shepherd J: Lipoprotein metabolism in hepatic lipase deficiency: Studies on the turnover of apolipoprotein B and on the effect of hepatic lipase on high density lipoprotein. *J Lipid Res* 29:1603–1611, 1988
36. Kashyap ML, Srivastava LS, Hynd BA, Brady D, Perisutti G, Glueck CJ, Gartside PS: Apolipoprotein CII and lipoprotein lipase in human nephrotic syndrome. *Atherosclerosis* 35:29–40, 1980
37. Breier CH, Lisch HJ, Braunsteiner H: Lipoproteine, Apolipoproteine, Lipoproteinlipase, hepatische Triglyceridlipase und Lecithin-Cholesterin-Acyl-Transferase bei Patienten mit nephrotischem Syndrom. *Schweiz Med Wschr* 113:909–913, 1983
38. Chan MK, Varghese Z, Persaud JW, Moorhead JF: Post-heparin hepatic and lipoprotein lipase activities in nephrotic syndrome. *Aust NZ J Med* 14:841–847, 1984
39. Calandra S, Gottardi E, Tarugi P: Plasma post-heparin lipolytic activity in rats with nephrotic syndrome. *Hormone Metab Res* 15:361, 1983
40. Furukawa S, Hirano T, Mamo JCL, Nagano S, Takahashi T: Catabolic defect of triglyceride is associated with abnormal very low density lipoprotein in experimental nephrosis. *Metabolism* 39:101–107, 1990
41. Shepherd J, Caslake M, Lorimer AR, Vallance BD, Packard CJ: Fenofibrate reduces low density lipoprotein catabolism in hypertriglyceridemic subjects. *Arteriosclerosis* 5:162–168, 1985
42. Goldberg ACK, Eliaschewitz FG, Quintao ECR: Origin of hypercholesterolemia in chronic experimental nephrotic syndrome. *Kidney Int* 12:23–27, 1977
43. McKenzie IFC, Nestel P: Studies on the turnover of trigylyceride and esterified cholesterol in subjects with the nephrotic syndrome. *J Clin Invest* 47:1685–1695, 1968
44. Pease RJ, Leiper JM: Regulation of hepatic apolipoprotein B-containing lipoprotein secretion. *Curr Opin Lipidol* 7:132–138, 1996
45. Taskinen MR, Packard CJ, Shepherd J: Effect of insulin therapy on the metabolic fate of apolipoprotein B-containing lipoproteins in NIDDM. *Diabetes* 39:1017–1027, 1990
46. Thompson GR, Naoumova RP, Watts GF: Role of cholesterol in regulating apolipoprotein B secretion by the liver. *J Lipid Res* 37:439–447, 1996
47. Katz J, Bonorris G, Okuyama S, Sellers AL: Albumin synthesis in perfused liver of normal and nephrotic rats. *Am J Physiol* 212:1255–1260, 1967
48. Jensen H, Rossing N, Andersen SB, Jarnum S: Albumin metabolism in the nephrotic syndrome in adults. *Clin Sci* 33:445–457, 1967
49. Baxter JH, Goodman HC, Allen JC: Effects of infusions of serum albumin on serum lipids and lipoproteins in nephrosis. *J Clin Invest* 40:490–497, 1961
50. Hirano T, Furukawa S, Kurokawa M, Ebara T, Dixon JL, Nagano S: Intracellualr apoprotein B degradation is suppressed by decreased albumin concentration in Hep G2 cells. *Kidney Int* 47:412–431, 1995

## APPENDIX I

Kinetics of free leucine and leucine prepared from apolipoprotein B from patients with nephrotic syndrome. Calculated values are given for k(i,j) and M(i), figures in italics denote fractional standard deviations.

| | WM | | KF | | JO | | HA | | MC | |
|---|---|---|---|---|---|---|---|---|---|---|
| k(0,1)[a] | 2.950 | *0.019* | 1.203 | *0.029* | 4.085 | *0.134* | 0.621 | *0.015* | 2.034 | *0.046* |
| k(3,1) | 5.829 | *0.027* | 1.823 | *0.039* | 5.858 | *0.176* | 2.745 | *0.011* | 2.576 | *0.039* |
| k(1,3) | 0.417 | *0.052* | 0.107 | *0.090* | 0.609 | *0.083* | 0.070 | *0.027* | 0.189 | *0.032* |
| k(4,3) | 0.416 | *0.027* | 0.257 | *0.063* | 0.568 | *0.038* | 0.162 | *0.030* | 0.262 | *0.028* |
| K(3,4) | 0.021 | *0.030* | 0.033 | *0.038* | 0.025 | *0.053* | 0.036 | *0.015* | 0.024 | *0.040* |
| k(2,1) | 6.923 | *0.050* | 3.213 | *0.081* | 6.803 | *0.149* | 2.888 | *0.019* | 9.044 | *0.105* |
| k(5,2) | 0.068 | *0.017* | 0.028 | *0.021* | 0.039 | *0.130* | 0.027 | *0.009* | 0.034 | *0.036* |

## APPENDIX I (CONTINUED)

| | WM | | KF | | JO | | HA | | MC | |
|---|---|---|---|---|---|---|---|---|---|---|
| P(1)[b] | 0.942 | *0.008* | 0.928 | *0.008* | 0.847 | *0.009* | 0.509 | *0.006* | 0.965 | *0.026* |
| d(9,5)[c] | 0.225 | *0.021* | 0.055 | *0.239* | 0.081 | *0.244* | 0.054 | *0.073* | 0.070 | *0.089* |
| d(12,5) | 0.024 | *0.086* | 0.098 | *0.029* | 0.039 | *0.165* | 0.142 | *0.016* | 0.053 | *0.028* |
| d(14,5) | 0.000 | — | 0.050 | *0.046* | 0.220 | *0.022* | 0.260 | *0.010* | 0.061 | *0.030* |
| k(7,6) | 1.140 | *0.071* | 10.00 | *>0.50* | 2.923 | *0.316* | 3.389 | *0.063* | 9.273 | *0.270* |
| k(0,7) | 0.060 | *0.074* | 0.161 | *0.298* | 0.073 | *0.266* | 0.100 | *>0.50* | 0.617 | *0.034* |
| k(8,6) | 0.000 | — | 0.346 | *0.053* | 0.057 | *0.272* | 0.010 | *>0.50* | 0.389 | *0.251* |
| k(0,8) | 0.000 | — | 0.092 | *0.042* | 0.025 | *0.063* | 1.000 | *>0.50* | 0.120 | *0.053* |
| k(9,7) | 0.111 | *0.030* | 1.103 | *0.025* | 0.641 | *0.039* | 1.387 | *0.020* | 0.404 | *0.017* |
| k(0,10) | 0.028 | *0.073* | 0.038 | *0.032* | 0.000 | — | 0.000 | — | 0.000 | — |
| k(12,10) | 0.053 | *0.021* | 0.076 | *0.018* | 0.077 | *0.029* | 0.107 | *0.004* | 0.080 | *0.016* |
| k(13,10) | 0.031 | *0.039* | 0.028 | *0.060* | 0.052 | *0.046* | 0.007 | *0.039* | 0.013 | *0.104* |
| k(14,12) | 0.231 | *0.047* | 0.112 | *0.037* | 0.084 | *0.049* | 0.157 | *0.014* | 0.069 | *0.023* |
| k(0,14) | 0.012 | *0.015* | 0.012 | *0.011* | 0.008 | *0.016* | 0.020 | *0.004* | 0.013 | *0.012* |
| k(14,15) | 0.002 | *0.214* | 0.000 | — | 0.000 | — | 0.000 | — | 0.000 | — |
| Q(1)[d] | 179 | | 569 | | 134 | | 413 | | 345 | |
| Q(2) | 177 | | 565 | | 133 | | 409 | | 343 | |
| Q(3) | 2499 | | 9667 | | 1291 | | 16310 | | 4701 | |
| Q(4) | 49238 | | 74601 | | 29796 | | 74096 | | 50602 | |
| U(1)[e] | 540 | | 700 | | 554 | | 268 | | 712 | |
| Q(6) | 7.9 | | 1.2 | | 1.2 | | 1.8 | | 1.0 | |
| Q(7) | 52.9 | | 9.6 | | 4.7 | | 4.0 | | 9.0 | |
| Q(8) | 8.7 | | 4.6 | | 2.6 | | 0.0 | | 3.2 | |
| M(VLDL-1)[f] | 69.5 | *70* | 15.4 | *15* | 8.5 | *8* | 5.8 | *6* | 13.2 | *13* |
| Q(9) | 7.5 | | 1.1 | | 1.2 | | 1.8 | | 0.5 | |
| Q(10) | 77.0 | | 78.2 | | 26.3 | | 53.9 | | 46.5 | |
| Q(11) | 8.3 | | 4.2 | | 2.6 | | 0.0 | | 1.5 | |
| M(VLDL-2) | 92.8 | *93* | 83.5 | *84* | 30.1 | *30* | 55.7 | *57* | 48.5 | *48* |
| Q(12) | 17.0 | | 49.9 | | 26.3 | | 46.7 | | 62.3 | |
| Q(13) | 56.3 | | 70.1 | | 68.5 | | 225.4 | | 41.9 | |
| M(IDL) | 73.3 | *73* | 120.0 | *120* | 94.8 | *95* | 272.1 | *271* | 104.2 | *106* |
| M(LDL)=Q(14) | 322.4 | *322* | 522.4 | *522* | 408.2 | *408* | 513.7 | *517* | 374.6 | *375* |
| Q(15) | 129.0 | | 208.9 | | 163.3 | | 205.5 | | 149.8 | |

| | EA | | ED | | HS | | KD | |
|---|---|---|---|---|---|---|---|---|
| k(0,1)[a] | 1.890 | *0.019* | 1.000 | *>0.50* | 2.077 | *0.070* | 1.222 | *0.034* |
| k(3,1) | 5.549 | *0.028* | 3.639 | *0.011* | 3.880 | *0.071* | 1.377 | *0.057* |
| k(1,3) | 0.271 | *0.056* | 0.022 | *0.017* | 0.047 | *0.081* | 0.303 | *>0.50* |
| k(4,3) | 0.418 | *0.030* | 0.029 | *0.024* | 0.070 | *0.074* | 0.574 | *0.101* |
| k(3,4) | 0.051 | *0.026* | 0.003 | *0.040* | 0.019 | *0.044* | 0.041 | *0.091* |
| k(2,1) | 3.403 | *0.019* | 2.302 | *0.016* | 2.500 | *>0.50* | 2.295 | *0.088* |
| k(5,2) | 0.043 | *0.018* | 0.024 | *0.009* | 0.059 | *0.062* | 0.026 | *0.024* |
| P(I)[b] | 0.850 | *0.008* | 0.603 | *0.008* | 0.929 | *0.008* | 0.900 | *0.010* |
| d(9,5)[c] | 0.273 | *0.009* | 0.228 | *0.014* | 0.230 | *0.024* | 0.193 | *0.053* |
| d(12,5) | 0.084 | *0.029* | 0.098 | *0.011* | 0.044 | *0.045* | 0.080 | *0.053* |
| d(14,5) | 0.253 | *0.016* | 0.040 | *0.023* | 0.046 | *0.049* | 0.004 | *>0.50* |
| k(7,6) | 1.912 | *0.059* | 0.225 | *0.009* | 10.00 | *>0.50* | 0.263 | *0.101* |
| k(0,7) | 0.000 | — | 0.001 | *>0.50* | 0.005 | *>0.50* | 0.000 | — |
| k(8,6) | 0.014 | *0.315* | 0.101 | *0.018* | 0.000 | — | 0.155 | *0.110* |
| k(0,8) | 0.142 | *0.271* | 0.813 | *0.088* | 0.000 | — | 0.500 | *>0.50* |
| k(9,7) | 2.256 | *0.063* | 2.952 | *0.103* | 0.122 | *0.042* | 0.469 | *0.163* |
| k(0,10) | 0.000 | — | 0.000 | — | 0.052 | *0.050* | 0.000 | — |
| k(12,10) | 0.261 | *0.008* | 0.162 | *0.015* | 0.055 | *0.026* | 0.043 | *0.035* |
| k(13,10) | 0.069 | *0.014* | 0.150 | *0.020* | 0.020 | *0.074* | 0.054 | *0.042* |
| k(14,12) | 0.575 | *0.017* | 0.134 | *0.016* | 0.134 | *0.055* | 0.110 | *0.049* |
| k(0,14) | 0.017 | *0.006* | 0.008 | *0.005* | 0.015 | *0.014* | 0.011 | *0.018* |
| k(14,15) | 0.000 | — | 0.000 | — | 0.000 | — | 0.000 | — |
| Q(1)[d] | 257 | | 272 | | 206 | | 685 | |
| Q(2) | 254 | | 269 | | 201 | | 677 | |
| Q(3) | 5274 | | 45888 | | 16875 | | 3108 | |
| Q(4) | 43350 | | 393074 | | 61785 | | 43841 | |
| U(1)[e] | 497 | | 279 | | 439 | | 854 | |
| Q(6) | 2.2 | | 12.6 | | 0.8 | | 30.1 | |
| Q(7) | 1.9 | | 1.0 | | 63.3 | | 16.9 | |
| Q(8) | 0.2 | | 1.6 | | 0.8 | | 9.3 | |
| M(VLDL-1)[f] | 4.3 | *4* | 15.2 | *16* | 66.5 | *67* | 56.3 | *58* |
| Q(9) | 3.7 | | 13.2 | | 1.0 | | 27.0 | |
| Q(10) | 21.5 | | 9.5 | | 81.7 | | 72.9 | |

## APPENDIX I (CONTINUED)

| | EA | | ED | | HS | | KD | |
|---|---|---|---|---|---|---|---|---|
| Q(11) | 0.4 | | 1.6 | | 1.0 | | 8.3 | |
| M(VLDL-2) | 25.6 | *25* | 24.3 | *25* | 83.7 | *84* | 108.2 | *108* |
| Q(12) | 11.3 | | 16.3 | | 26.8 | | 41.0 | |
| Q(13) | 84.9 | | 48.8 | | 40.5 | | 81.2 | |
| M(IDL) | 96.2 | *97* | 65.1 | *66* | 67.3 | *67* | 122.2 | *122* |
| M(LDL)=Q(14) | 538.0 | *540* | 276.6 | *276* | 277.2 | *270* | 405.6 | *406* |
| Q(15) | 215.2 | | 110.6 | | 110.9 | | 162.4 | |

[a] k(i,j) describe the transfer of leucine from compartment j to i as fraction of pool j per hour. Fractional standard deviations (FSD) are given in parenthesis. k(i,j) values are transformed into kinetic transfer rates of apolipoprotein B from compartment j to i per day by multiplication with a constant factor (that is times 24 hours divided through 0.1212, the fractional leucine content of apo B). For model constraints see Methods.

[b] P(1) is a dilutional factor calculated to determine the apo B precursor pool enrichment in compartment 5. It is mathematically equivalent to U(5). For details see Methods and [24].

[c] d(i,5) describe the distribution of material leaving the delay compartment (compartment 5, Fig. 2) and entering the apo B compartments 6, 9, 12 and 14. d(6,5) + d(9,5) + d(12,5) + d(14,5) = 1.00.

[d] Q(i) is the leucine mass (mg) in compartment i. M(i) can be transformed into corresponding apo B masses by division through 0.1212 (i.e. the fractional leucine content of apo B).

[e] U(1) is the calculated tracee (i.e. native leucine) input into compartment 1.

[f] For M(VLDL-1), M(VLDL-2), M(IDL), and M(LDL) calculated and directly measured masses are given in italics.

## APPENDIX II

Kinetics of free leucine and leucine prepared from apolipoprotein B from normolipidemic controls. Calculated values are given for k(i,j) and M(i), figures in italics denote fractional standard deviations.

| | BG | | CE | | MM | | BJ | | RB | |
|---|---|---|---|---|---|---|---|---|---|---|
| k(0,1)[a] | 2.227 | *0.030* | 2.068 | *0.016* | 2.402 | *0.016* | 1.267 | *0.006* | 1.491 | *0.004* |
| k(3,1) | 2.780 | *0.024* | 4.845 | *0.010* | 1.654 | *0.057* | 1.477 | *0.007* | 2.611 | *0.005* |
| k(1,3) | 0.047 | *0.034* | 0.770 | *0.017* | 0.129 | *0.046* | 0.235 | — | 0.344 | *0.004* |
| k(4,3) | 0.024 | *0.041* | 0.592 | *0.012* | 2.500 | — | 0.172 | — | 0.510 | *0.003* |
| k(3,4) | 0.009 | *0.012* | 0.021 | *0.026* | 0.250 | — | 0.017 | — | 0.051 | *0.003* |
| k(2,1) | 3.131 | *0.035* | 2.500 | — | 2.500 | — | 2.500 | — | 2.500 | — |
| k(5,2) | 0.026 | *0.012* | 0.018 | *0.014* | 0.016 | *0.016* | 0.014 | *0.003* | 0.031 | *0.004* |
| P(1)[b] | 0.817 | *0.005* | 0.844 | *0.004* | 0.673 | *0.005* | 0.532 | *0.001* | 0.556 | *0.001* |
| d(9,5)[c] | 0.147 | *0.054* | 0.045 | *0.185* | 0.100 | *>0.50* | 0.020 | *>0.50* | 0.061 | *0.026* |
| d(12,5) | 0.035 | *0.011* | 0.059 | *0.028* | 0.379 | *0.009* | 0.038 | *0.012* | 0.142 | *0.005* |
| d(14,5) | 0.136 | *0.025* | 0.022 | *0.049* | 0.210 | *0.009* | 0.172 | *0.006* | 0.055 | *0.005* |
| k(7,6) | 5.000 | *>0.50* | 2.262 | *0.009* | 10.00 | *>0.50* | 0.513 | *0.005* | 10.00 | — |
| k(0,7) | 0.000 | — | 14.23 | *0.270* | 0.635 | *>0.50* | 1.464 | *0.012* | 0.640 | *0.008* |
| k(8,6) | 0.000 | — | 0.100 | *>0.50* | 0.413 | *0.054* | 0.002 | *0.009* | 0.401 | *0.063* |
| k(0,8) | 0.000 | — | 0.070 | *0.024* | 0.099 | *0.042* | 0.001 | *0.008* | 0.286 | *0.035* |
| k(9,7) | 0.667 | *0.015* | 9.603 | *0.274* | 0.839 | *0.021* | 0.626 | *0.007* | 0.590 | *0.009* |
| k(0,10) | 0.030 | *0.072* | 0.000 | — | 0.000 | — | 0.000 | — | 0.000 | — |
| k(12,10) | 0.376 | *0.009* | 0.181 | *0.010* | 0.131 | *0.119* | 0.600 | *>0.50* | 0.199 | *0.003* |
| k(13,10) | 0.019 | *0.045* | 0.074 | *0.024* | 0.004 | *0.236* | 0.000 | — | 0.070 | *0.008* |
| k(14,12) | 0.125 | *0.020* | 0.366 | *0.024* | 0.172 | *0.008* | 0.058 | *0.002* | 0.219 | *0.004* |
| k(0,14) | 0.020 | *0.013* | 0.012 | *0.008* | 0.026 | *0.006* | 0.018 | *0.002* | 0.027 | *0.002* |
| k(14,15) | 0.008 | *0.105* | 0.000 | — | 0.000 | — | 0.003 | *0.021* | 0.007 | *0.018* |
| Q(1)[d] | 222 | | 375 | | 470 | | 588 | | 607 | |
| Q(2) | 220 | | 373 | | 467 | | 585 | | 600 | |
| Q(3) | 13055 | | 2361 | | 6038 | | 3695 | | 4609 | |
| Q(4) | 36314 | | 66910 | | 60377 | | 36946 | | 46087 | |
| U(1)[e] | 500 | | 783 | | 1135 | | 753 | | 924 | |
| Q(6) | 0.8 | | 2.5 | | 0.2 | | 1.9 | | 1.3 | |
| Q(7) | 5.9 | | 0.2 | | 1.5 | | 3.0 | | 10.6 | |
| Q(8) | 0.8 | | 3.6 | | 1.0 | | 0.8 | | 1.8 | |
| M(VLDL-1)[f] | | | | | | | | | | |
| Q(9) | 1.0 | | 1.1 | | 0.2 | | 3.9 | | 0.7 | |
| Q(10) | 11.5 | | 9.8 | | 14.2 | | 3.4 | | 27.5 | |
| Q(11) | 1.0 | | 1.6 | | 0.9 | | 8.6 | | 0.0 | |
| M(VLDL-2) | | | | | | | | | | |
| Q(12) | 29.3 | | 6.0 | | 27.2 | | 39.7 | | 36.9 | |
| Q(13) | 11.5 | | 24.9 | | 37.5 | | 0.0 | | 61.4 | |
| M(IDL) | | | | | | | | | | |
| M(LDL)=Q(14) | 223.5 | | 188.9 | | 237.8 | | 209.7 | | 334.0 | |
| Q(15) | 89.4 | | 75.6 | | 95.1 | | 83.9 | | 133.6 | |

## APPENDIX II (CONTINUED)

| | CS | | WS | | JD | | GH | |
|---|---|---|---|---|---|---|---|---|
| k(0,1)[a] | 0.730 | *0.038* | 1.362 | *0.012* | 1.111 | *0.010* | 2.008 | *0.008* |
| k(3,1) | 1.573 | *0.023* | 1.455 | *0.010* | 1.618 | *0.007* | 2.175 | *0.010* |
| k(1,3) | 0.016 | *0.056* | 0.092 | *0.022* | 0.093 | *0.020* | 0.187 | *0.015* |
| k(4,3) | 0.030 | *0.013* | 0.162 | *0.013* | 0.173 | *0.016* | 0.286 | *0.010* |
| k(3,4) | 0.019 | *0.014* | 0.016 | *0.013* | 0.017 | *0.016* | 0.029 | *0.010* |
| k(2,1) | 13.16 | *0.093* | 2.543 | *0.022* | 2.389 | *0.012* | 2.610 | *0.013* |
| k(5,2) | 0.014 | *0.016* | 0.017 | *0.012* | 0.015 | *0.005* | 0.030 | *0.012* |
| P(1)[b] | 0.752 | *0.010* | 0.667 | *0.002* | 0.587 | *0.002* | 0.676 | *0.005* |
| d(9,5)[c] | 0.153 | *0.042* | 0.169 | *0.019* | 0.104 | *0.033* | 0.150 | *>0.50* |
| d(12,5) | 0.107 | *0.025* | 0.025 | *0.072* | 0.108 | *0.014* | 0.191 | *0.015* |
| d(14,5) | 0.157 | *0.020* | 0.000 | — | 0.189 | *0.007* | 0.007 | *0.036* |
| k(7,6) | 1.812 | *0.074* | 10.00 | *>0.50* | 7.000 | *>0.50* | 25.05 | *0.075* |
| k(0,7) | 0.000 | — | 0.660 | *0.031* | 0.500 | *>0.50* | 1.000 | *>0.50* |
| k(8,6) | 0.017 | *0.013* | 0.007 | *>0.50* | 3.537 | *0.013* | 0.000 | — |
| k(0,8) | 0.020 | *0.013* | 0.028 | *>0.50* | 1.043 | *0.010* | 0.000 | — |
| k(9,7) | 0.636 | *0.020* | 0.497 | *0.016* | 5.000 | *>0.50* | 1.200 | *>0.50* |
| k(0,10) | 0.160 | *0.022* | 0.000 | — | 0.000 | — | 0.205 | *0.017* |
| k(12,10) | 0.256 | *0.023* | 0.246 | *0.006* | 0.235 | *0.004* | 0.361 | *0.010* |
| k(13,10) | 0.014 | *0.050* | 0.096 | *0.012* | 0.004 | *0.082* | 0.035 | *0.020* |
| k(14,12) | 0.221 | *0.028* | 0.454 | *0.011* | 0.109 | *0.006* | 0.324 | *0.015* |
| k(0,14) | 0.024 | *0.014* | 0.011 | *0.003* | 0.021 | *0.003* | 0.016 | *0.006* |
| k(14,15) | 0.004 | *0.014* | 0.000 | — | 0.000 | — | 0.000 | — |
| Q(1)[d] | 567 | | 520 | | 470 | | 279 | |
| Q(2) | 567 | | 518 | | 467 | | 276 | |
| Q(3) | 54504 | | 8224 | | 8152 | | 3248 | |
| Q(4) | 86400 | | 82236 | | 81524 | | 32479 | |
| U(1)[e] | 422 | | 718 | | 529 | | 568 | |
| Q(6) | 2.6 | | 0.7 | | 0.4 | | 0.2 | |
| Q(7) | 7.4 | | 6.2 | | 0.5 | | 2.4 | |
| Q(8) | 2.2 | | 0.2 | | 1.3 | | 0.2 | |
| M(VLDL-1)[f] | 12.2 | *12* | 7.1 | *7* | 2.2 | *3* | 2.8 | *3* |
| Q(9) | 3.3 | | 0.5 | | 0.3 | | 0.2 | |
| Q(10) | 13.7 | | 13.4 | | 9.0 | | 6.9 | |
| Q(11) | 2.7 | | 0.1 | | 1.0 | | 0.2 | |
| M(VLDL-2) | 19.7 | *20* | 14.0 | *14* | 10.3 | *8* | 7.3 | *8* |
| Q(12) | 11.5 | | 7.8 | | 26.4 | | 7.7 | |
| Q(13) | 13.7 | | 45.8 | | 9.0 | | 25.8 | |
| M(IDL) | 25.2 | *26* | 53.6 | *55* | 35.4 | *37* | 33.5 | *35* |
| M(LDL)=Q(14) | 162.0 | *162* | 312.3 | *314* | 200.1 | *206* | 158.3 | *157* |
| Q(15) | 64.8 | | 124.9 | | 80.1 | | 63.3 | |

For footnotes see Appendix I.

## APPENDIX III

Kinetics of free leucine and leucine prepared from albumin from patients with nephrotic syndrome. Calculated values are given for k(i,j) and M(i), figures in italics denote fractional standard deviations.

| | WM | | KF | | JO | | HA | | MC | |
|---|---|---|---|---|---|---|---|---|---|---|
| k(0,1)[a] | 2.5944 | *0.016* | 1.1519 | *0.041* | 3.8419 | *0.016* | 0.5000 | *>0.50* | 0.5822 | *0.023* |
| k(3,1) | 14.899 | *0.062* | 1.4673 | *0.039* | 5.2488 | *0.060* | 2.8305 | *0.012* | 2.5057 | *0.017* |
| k(1,3) | 2.6566 | *0.092* | 0.0583 | *0.091* | 0.3254 | *0.241* | 0.0274 | *0.042* | 0.0640 | *0.061* |
| k(4,3) | 0.5576 | *0.050* | 0.1185 | *0.097* | 0.4105 | *0.111* | 0.0365 | *0.086* | 0.1776 | *0.056* |
| k(3,4) | 0.0122 | *0.041* | 0.0267 | *0.055* | 0.0169 | *0.104* | 0.0101 | *0.084* | 0.0254 | *0.027* |
| k(2,1) | 1.1074 | *>0.50* | 2.3958 | *0.115* | 2.5000 | *>0.50* | 0.7694 | *0.040* | 2.5000 | *>0.50* |
| k(5,2) | 1.0888 | *0.033* | 0.2793 | *0.034* | 0.4245 | *0.027* | 0.2699 | *0.031* | 0.1245 | *0.020* |
| P(1)[b] | 0.942 | | 0.928 | | 0.847 | | 0.509 | | 0.965 | |
| k(7,6) | 0.0055 | *0.099* | 0.0200 | *>0.50* | 0.0001 | *0.100* | 0.0193 | *0.118* | 0.0071 | *0.027* |
| k(6,7) | 0.0017 | *0.213* | 0.0223 | *0.096* | 0.0001 | *0.100* | 0.0108 | *0.166* | 0.0003 | *0.316* |
| k(0,6) | 0.0083 | *0.015* | 0.0091 | *0.024* | 0.0063 | *0.024* | 0.0085 | *0.016* | 0.0059 | *0.017* |
| Q(1)[c] | 179 | | 446 | | 145 | | 421 | | 460 | |
| Q(2) | 90 | | 399 | | 139 | | 312 | | 454 | |
| Q(3) | 1003 | | 11219 | | 2338 | | 43489 | | 18017 | |
| Q(4) | 46002 | | 49890 | | 56769 | | 157313 | | 126154 | |
| U(1)[d] | 562 | *12100* | 625 | | 616 | | 85 | | 325 | |
| M(Alb.)=Q(6) | 11802 | | 12296 | *12880* | 9350 | *9408* | 9889 | *10100* | 9687 | *9740* |
| Q(7) | 38050 | | 11048 | | 9350 | | 17680 | | 226578 | |

## APPENDIX III (CONTINUED)

| | EA | | ED | | HS | | KD | |
|---|---|---|---|---|---|---|---|---|
| k(0,1)[a] | 0.5000 | *>0.50* | 0.2444 | *>0.50* | 0.8030 | *0.076* | 0.7172 | *0.005* |
| k(3,1) | 4.6795 | *0.019* | 3.3297 | *0.003* | 3.2329 | *0.031* | 2.5130 | *0.010* |
| k(1,3) | 0.0853 | *0.033* | 0.0089 | *0.007* | 0.0851 | *0.113* | 0.2389 | *0.010* |
| k(4,3) | 0.3507 | *0.020* | 0.0180 | *0.008* | 0.1303 | *0.090* | 0.5789 | *0.024* |
| k(3,4) | 0.0314 | *0.025* | 0.0037 | *0.008* | 0.0158 | *0.071* | 0.0579 | *0.030* |
| k(2,1) | 2.8261 | *0.029* | 0.9264 | *0.013* | 2.5000 | *>0.50* | 0.3250 | *>0.50* |
| k(5,2) | 0.4101 | *0.028* | 0.2215 | *0.007* | 0.2869 | *0.032* | 0.2844 | *0.023* |
| P(1)[b] | 0.850 | | 0.603 | | 0.929 | | 0.900 | |
| k(7,6) | 0.0001 | *0.100* | 0.0113 | *0.032* | 0.0058 | *0.147* | 0.0434 | *0.014* |
| k(6,7) | 0.0001 | *0.100* | 0.0132 | *0.032* | 0.0034 | *0.361* | 0.0062 | *0.015* |
| k(0,6) | 0.0094 | *0.012* | 0.0072 | *0.004* | 0.0050 | *0.019* | 0.0051 | *0.008* |
| Q(1)[c] | 133 | | 270 | | 300 | | 705 | |
| Q(2) | 116 | | 218 | | 292 | | 376 | |
| Q(3) | 7291 | | 101629 | | 11402 | | 7417 | |
| Q(4) | 81510 | | 501642 | | 94195 | | 74166 | |
| U(1)[d] | 114 | | 114 | | 325 | | 613 | |
| M(A1b.)=Q(6) | 5062 | *5170* | 6701 | *6800* | 16669 | *16900* | 21099 | *20850* |
| Q(7) | 5072 | | 5720 | | 28581 | | 147243 | |

[a] k(i,j) values are transformed into kinetic transfer rates of albumin from compartment j to i per day by multiplication with a constant factor (that is times 24 hours divided through 0.1200, the fractional leucine content of albumin).

[b] P(1) is the dilutional factor which defines the hepatic precursor pool enrichment in compartment 5. It was determined in the apo B turnover studies documented in Appendix I. For details see Methods.

[c] Q(i) is the leucine mass (mg) in compartment i. Q(6) can be transformed into corresponding albumin pools by division through 0.1200 (i.e. the fractional leucine content of albumin). Measured albumin pools are given in italics.

[d] U(1) is the calculated tracee (i.e. native leucine) input into compartment 1.

## APPENDIX IV

Kinetics of free leucine and leucine prepared from albumin from normal controls. Calculated values are given for k(i,j) and M(i), figures in italics denote fractional standard deviations.

| | CE | | RB | | CS | | WS | | JD | | GH | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| k(0,1)[a] | 0.6386 | *0.154* | 0.6677 | *0.022* | 0.3000 | *>0.50* | 1.7625 | *0.011* | 1.6331 | *0.016* | 2.2635 | *0.008* |
| k(3,1) | 4.7563 | *0.031* | 3.0626 | *0.010* | 0.9465 | *0.039* | 1.3797 | *0.045* | 2.1374 | *0.031* | 1.0911 | *0.025* |
| k(1,3) | 0.1840 | *0.071* | 0.0937 | *0.038* | 0.0066 | *0.061* | 0.1775 | *0.124* | 0.1399 | *0.062* | 0.0857 | *0.059* |
| k(4,3) | 0.3796 | *0.037* | 0.2721 | *0.031* | 0.0090 | *0.105* | 0.1471 | *0.082* | 0.2217 | *0.038* | 0.1101 | *0.071* |
| k(3,4) | 0.0155 | *0.048* | 0.0424 | *0.022* | 0.0027 | *0.109* | 0.0174 | *0.052* | 0.0158 | *0.041* | 0.0296 | *0.040* |
| k(2,1) | 4.7900 | *0.082* | 2.1585 | *0.024* | 0.4644 | *0.084* | 6.5994 | *0.188* | 4.2957 | *0.097* | 2.5000 | *<0.50* |
| k(5,2) | 0.1931 | *0.034* | 0.1007 | *0.010* | 0.0551 | *0.048* | 0.0847 | *0.034* | 0.1530 | *0.037* | 0.1268 | *0.036* |
| P(1)[b] | 0.844 | | 0.556 | | 0.752 | | 0.667 | | 0.587 | | 0.676 | |
| k(7,6) | 0.0100 | *0.139* | 0.0125 | *0.026* | 0.0100 | *0.100* | 0.0047 | *0.156* | 0.0128 | *0.126* | 0.0170 | *0.118* |
| k(6,7) | 0.0035 | *0.226* | 0.0011 | *0.118* | 0.0109 | *0.092* | 0.0027 | *0.362* | 0.0109 | *0.139* | 0.0114 | *0.128* |
| k(0,6) | 0.0034 | *0.022* | 0.0028 | *0.008* | 0.0028 | *0.032* | 0.0021 | *0.021* | 0.0038 | *0.025* | 0.0025 | *0.024* |
| Q(1)[c] | 250 | | 655 | | 1005 | | 475 | | 303 | | 291 | |
| Q(2) | 240 | | 625 | | 899 | | 469 | | 293 | | 277 | |
| Q(3) | 6462 | | 21403 | | 143736 | | 3692 | | 4629 | *3706* | | |
| Q(4) | 15705 | | 137272 | | 483350 | | 31263 | | 65048 | | 13800 | |
| U(1)[d] | 206 | | 500 | | 351 | | 877 | | 540 | | 694 | |
| M(A1b.)=Q(6) | 13815 | *14100* | 22440 | *23010* | 17826 | *12150* | 19101 | *19300* | 11753 | *12150* | 14084 | *14300* |
| Q(7) | 39207 | | 251780 | | 16408 | | 32722 | | 13854 | *20935* | | |

## APPENDIX V

Abbreviations used in this article are apo B, apolipoprotein B; FCR, fractional catabolism rate; FER, fractional elimination rate; FSD, fractional standard deviation; GC-MS, gas chromatography mass spectrometry; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein.